ELSEVIER

European Journal of Pharmacology 437 (2002) 69–72

www.elsevier.com/locate/ejphar

Short communication

Role of K^+ channels in the PACAP-induced catecholamine secretion from the rat adrenal gland

Yasuo Fukushima [a], Takahiro Nagayama [a], Hirohiko Hikichi [a], Kazuhiko Mizukami [a], Makoto Yoshida [a], Mizue Suzuki-Kusaba [a], Hiroaki Hisa [a,*], Tomohiko Kimura [b], Susumu Satoh [a]

[a] *Laboratory of Pharmacology, Graduate School of Pharmaceutical Sciences, Tohoku University, Aobayama, Sendai, 980-8578, Japan*
[b] *Department of Dental Pharmacology, The Nippon Dental University School of Dentistry at Niigata, Hamaura-cho, Niigata, 951-8580, Japan*

Received 5 December 2001; accepted 11 January 2002

Abstract

We eluciated whether K^+ channels modulate adrenal catecholamine secretion induced by pituitary adenylate cyclase-activating polypeptide (PACAP) in the isolated perfused rat adrenal gland. PACAP (100 nM) increased adrenal epinephrine output. The PACAP-induced responses were enhanced by treatment with apamin (10–100 nM) in a concentration-dependent manner. In the presence of nifedipine (3 μM), apamin (1 μM) did not enhance the PACAP-induced responses. Charybdotoxin (1–100 nM) had little influence on the PACAP-induced responses. These results suggest that small-conductance Ca^{2+}-activated K^+ channels interfere with L-type voltage-dependent Ca^{2+} channels to counteract the PACAP-induced adrenal catecholamine secretion. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: PACAP (pituitary adenylate cyclase-activating polypeptide); Small-conductance Ca^{2+}-activated K^+ (SK_{Ca}) channel; Large-conductance Ca^{2+}-activated K^+ (SK_{Ca}) channel; Adrenal gland, rat; Catecholamine secretion

1. Introduction

Pituitary adenylate cyclase-activating polypeptide (PACAP), originally isolated from ovine hypothalamus (Miyata et al., 1989), exists in the adrenal gland (Ghatei et al., 1993) and has been considered to participate in the control of adrenal catecholamine secretion.

PACAP increases intracellular free Ca^{2+} and evokes catecholamine secretion in rat (Przywara et al., 1996) and bovine (Tanaka et al., 1996) cultured adrenal chromaffin cells. The PACAP-induced elevation of intracellular free Ca^{2+}, which is an essential step for the catecholamine secretion, is suggested to result from Ca^{2+} release from intracellular Ca^{2+} store and Ca^{2+} influx via L-type voltage-dependent Ca^{2+} channels (Tanaka et al., 1996), and N- and Q-type Ca^{2+} channels may also participate in the PACAP-induced catecholamine secretion (O'Farrell and Marley, 1997) in bovine adrenal chromaffin cells. We have recently found that treatment with an L-type Ca^{2+} channel blocker nifedipine, but not an N-type Ca^{2+} channel blocker ω-conotoxin GVIA or a P/Q-type Ca^{2+} channel blocker ω-conotoxin MVIIC, attenuated catecholamine secretion induced by PACAP in the isolated perfused rat adrenal gland (Fukushima et al., 2001). Processes of the PACAP-induced cathecholamine secretion may thus involve activation of L-type voltage-dependent Ca^{2+} channels in the rat adrenal gland.

The elevation of intracellular free Ca^{2+} may open Ca^{2+}-activated K^+ (K_{Ca}) channels on adrenal chromaffin cells. Opening of the K^+ channels leads the membrane potential to hyperpolarization, which could interfere with opening of voltage-dependent Ca^{2+} channels and thereby counteract adrenal catecholamine secretion. Previous reports have demonstrated that the blockade of K_{Ca} channels enhanced catecholamine secretion in response to cholinergic stimuli in bovine adrenal chromaffin cells (Wada et al., 1995) and the isolated perfused rat adrenal adrenal gland (Nagayama et al., 2000b).

However, it has been unknown whether the K^+ channels modulate the PACAP-induced adrenal cathecholamine secretion. To clarify this issue, in the present study we examined the effects of the small-conductance K_{Ca} (SK_{Ca}) channel blocker apamin (Kawai and Watanabe, 1986) and a large-conductance K_{Ca} (BK_{Ca}) channel and intermediate-

* Corresponding author. Tel.: +81-22-217-6837; fax: +81-22-217-6835.
E-mail address: hhisa@mail.pharm.tohoku.ac.jp (H. Hisa).

0014-2999/02/$ - see front matter
PII: S0014-2999(02)01275-X

conductance K_{Ca} (IK_{Ca}) channel blocker charybdotoxin (Neylon et al., 1999) on epinephrine and norepinephrine secretion in response to PACAP in the isolated perfused rat adrenal gland.

2. Materials and methods

2.1. Preparation

All procedures for handling animals were approved by the Animal Experimentation Committee of Tohoku University Graduate School of Pharmaceutical Sciences. Male Wistar rats, weighing 230–330 g, were anesthetized with pentobarbital Na (50 mg/kg, i.p.). The surgical procedure was the same previously described (Nagayama et al., 1999). A polyethylene catheter for perfusion of the adrenal gland was inserted into the adrenal vein through the renal vein. Then the adrenal gland was removed from the animal, and a small slit was made into the adrenal cortex just opposite the entrance of the adrenal vein. Retrograde perfusion of the adrenal gland was started, and the adrenal gland was placed in a water-jacketed chamber that was maintained at 37 °C with thermostatically controlled water circulator (NTT-1200, EYELA, Tokyo, Japan). The perfussion was carried out by means of a peristaltic pump (MP-3A, EYELA) at a rate of 0.2 ml/min with Krebs–Henseleit solution that was maintained at 37 °C and bubbled with a mixture of 95% O_2 and 5% CO_2. After extraction of the adrenal gland, the animal was killed by exsanguination.

2.2. PACAP infusion

PACAP (PACAP27, Peptide Institute, Osaka, Japan) was infused into the perfusion stream through a branching catheter by using a microsyringe pump (CMA/200, Bioanalytical Systems, West Lafayette, IN, USA) at a rate of 0.02 ml/min for 3 min in three or four consecutive experimental periods. The calculated concentration of PACAP in the perfusate was 100 nM. Before the start of experiments, infusion of 100-nM PACAP was twice performed for 5 min at 10-min intervals to obtain stable catecholamine output responses as previously described (Fukushima et al., 2001). After these conditioning infusion periods, catecholamine secretion responses induced by 100-nM PACAP for 3 min were stable in four consecutive experimental periods (Fukushima et al., 2001).

2.3. Experimental protocol

The first infusion of PACAP (after the conditioning infusion periods) was regulated as control (first trial). In Groups 1 and 2, perfusion with Krebs–Henseleit solution containing apamin (Peptide Institute) at 10, 100 and 1000 nM (Group 1, $n=9$) or charybdotoxin (Peptide Institute) at 1, 10 and 100 nM (Group 2, $n=9$) was started 5 min before the start of the second, third and fourth trials, respectively. In Group 3 ($n=6$), perfusion with nifedipine (3 mM; Sigma, St. Louis, MO, USA)-containing and both nifedipine (3 mM)- and apamin (1 mM)-containing Krebs–Henseleit solution was started 5 min before the start of the second and third trials, respectively.

2.4. Perfusate sampling and catecholamine output determination

Perfusate was collected for 60 s before the PACAP infusion and for 200 s beginning at the start of the PACAP infusion. As an internal standard for catecholamines, 25 ng of 3,4-dihydroxybenzylamine, contained in 50 ml of 0.1-M perchloric acid, was added to each perfusate sample. Catecholamines in perfusate samples were measured by high-performance liquid chromatography with electrochemical detection as previously described (Fukushima et al., 2001). Epinephrine and norepinephrine output (nmol/min) were calculated by multiplying the perfusate catecholamine

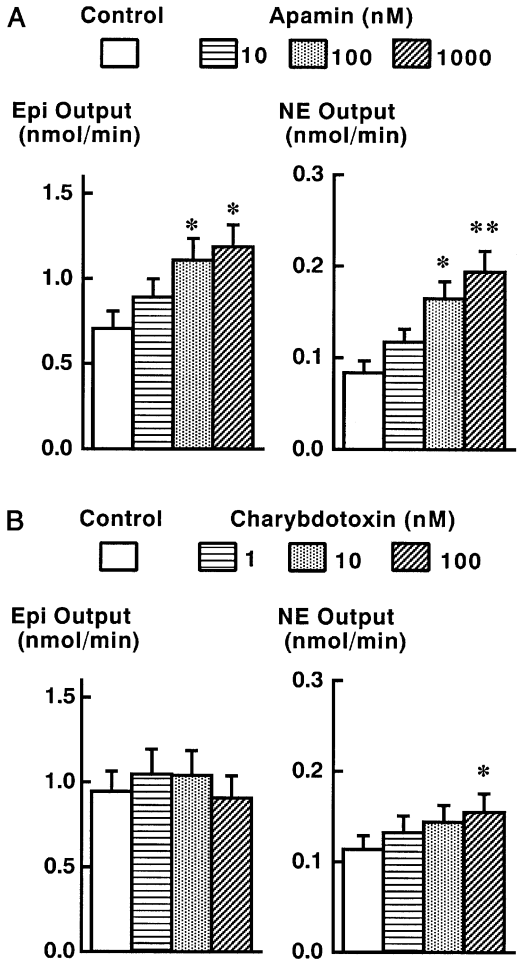

Fig. 1. Effects of apamin (A, Group 1, $n=9$) and charybdotoxin (B, Group 2, $n=9$) on PACAP-induced increases in epinephrine (Epi) and norepinephrine (NE) output. Values are means ± S.E.M. * $P<0.05$, ** $P<0.01$ compared with corresponding control values (Dunnett's test).

concentration (nmol/ml) by the perfusion rate (0.2 ml/min). The PACAP-induced increases in catecholamine output were calculated by subtracting catecholamine output before the PACAP infusion from that obtained during the PACAP infusion.

2.5. Data analysis

The results are expressed as means ± S.E.M. Single-factor analysis of variance for repeated measures was used for statistical analysis, and Dunnett's test or Scheffé's test was applied for multiple comparison. $P < 0.05$ were considered to be statistically significant.

3. Results

Infusion of PACAP increased epinephrine and norepinephrine output in all experimental groups. Treatment with apamin (100 and 1000 nM) significantly enhanced the PACAP-induced increases in epinephrine output (Group 1, Fig. 1A). Percentages of the enhancement by apamin of the epinephrine and norepinephrine output responses were about 170% and 200% to the control values, respectively. Apamin did not affect basal catecholamine output (obtained before the PACAP infusion); the values in the control and apamin treatment (10, 100 and 1000 nM) periods were 0.21 ± 0.03, 0.18 ± 0.03, 0.19 ± 0.02 and 0.20 ± 0.04 nmol/min in epinephrine output and 0.026 ± 0.005, 0.023 ± 0.005, 0.024 ± 0.002 and 0.026 ± 0.002 nmol/min in norepinephrine output, respectively.

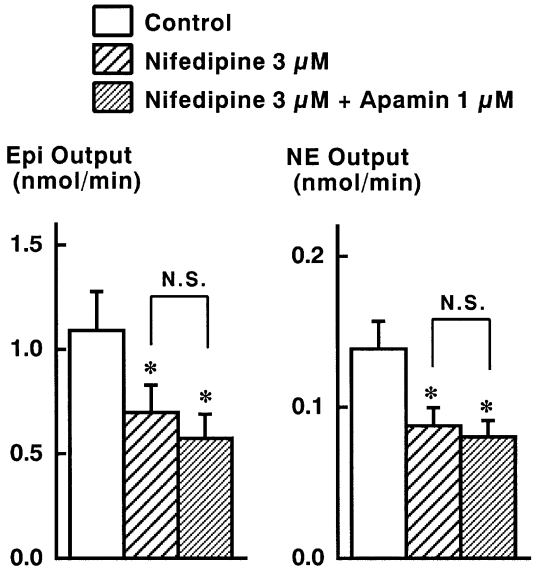

Fig. 2. Effects of apamin on PACAP-induced increases in epinephrine (Epi) and norepinephrine (NE) output in the presence of nifedipine (Group 3, $n = 6$). Values are means ± S.E.M. * $P < 0.01$ compared with corresponding control values obtained before the nifedipine treatment, and "N.S." indicates there was no statistically significant differences between the values (Scheffé's test).

Treatment with charybdotoxin (100 nM) also enhanced the PACAP-induced norepinephrine output response (Group 2, Fig. 1B), but the enhancement (about 130% to the control value) was smaller than that obtained with apamin. Charybdotoxin did not affect the PACAP-induced increase in epinephrine output.

Nifedipine (3 mM) attenuated the PACAP-induced increases in epinephrine and norepinephrine output (Group 3, Fig. 2). In the presence of nifedipine, apamin (1 mM) failed to enhance the PACAP-induced increases in epinephrine and norepinephrine output (Fig. 2). The values of basal cathecholamine output in the control, nifedipine treatment, and nifedipine and apamin treatment periods were 0.19 ± 0.03, 0.15 ± 0.01 and 0.12 ± 0.01 nmol/min in epinephrine output and 0.015 ± 0.003, 0.015 ± 0.003 and 0.012 ± 0.002 nmol/min in norepinephrine output, respectively.

4. Discussion

The infusion of PACAP into the isolated adrenal gland increased epinephrine and norepinephrine output, the responses of which were enhanced by treatment with the SK_{Ca} channel blocker apamin in a concentration-dependent manner. This finding suggests that SK_{Ca} channels are activated to counteract the PACAP-induced catecholamine secretion in the rat adrenal gland. Apamin treatment itself did not increase catecholamine output, indicating that apamin interferes with catecholamine secretion processes activated by PACAP. In the previous study (Nagayama et al., 2000b), apamin did not affect the epinephrine output response induced by transmural electrical stimulation whereas it enhanced both the epinephrine and norepinephrine output responses induced by exogenous acetylcholine in the isolated perfused rat adrenal gland. Apamin therefore does not seem to cause a non-specific enhancement of the catecholamine secretion. The present study is the first that revealed an inhibitory role of K_{Ca} channels in the PACAP-induced adrenal catecholamine secretion.

Although the BK_{Ca} and IK_{Ca} channel blocker charybdotoxin enhanced the PACAP-induced norepinephrine output response, the effect was smaller that that of apamin and charybdotoxin failed to enhance the PACAP-induced epinephrine output response. The concentration range of charybdotoxin seems to be sufficient, because 100-nM charybdotoxin significantly enhanced the electrical stimulation-induced norepinephrine output response in the rat adrenal gland (Nagayama et al., 2000b). BK_{Ca} or IK_{Ca} channels may therefore play little role in the PACAP-induced adrenal catecholamine secretion.

In additional experiments, neither a K_A-channel blocker mast cell degranulating peptide (Stansfeld et al., 1987), which resembles apamin in the secondary structure, nor a K_V channel blocker margatoxin (Bednarek et al., 1994) affected the PACAP-induced catecholamine output responses (data not shown). These voltage-dependent K^+ chan-

nels may play no role in the PACAP-induced adrenal catecholamine secretion.

It has been suggested that L-type Ca^{2+} channels participate in the PACAP-induced catecholamine secretion in the cultured bovine adrenal chromaffin cells (Tanaka et al., 1996), the perfused rat adrenal gland (Fukushima et al., 2001) and the dog adrenal gland in vivo (Geng et al., 1997). A study performed in our laboratory demonstrated that contribution of L-type Ca^{2+} channels to catecholamine secretion during muscarinic stimulation is cancelled by concomitantly activated SK_{Ca} channels in the isolated rat adrenal gland (Nagayama et al, 2000a). Considering this modulation on L-type Ca^{2+} channels in the present study, we also examined the effects of apamin in the presence of an L-type Ca^{2+} channel blocker nifedipine.

Treatment with nifedipine blunted the PACAP-induced epinephrine and norepinephrine output responses, as we have recently reported (Fukushima et al., 2001). In the presence of nifedipine, apamin failed to enhance the PACAP-induced catecholamine output responses. This result indicates that the enhancement by apamin requires activation of L-type Ca^{2+} channels. It is therefore likely that PACAP opens SK_{Ca} channels by elevating intracellular Ca^{2+}, and K^+ efflux through SK_{Ca} channels attenuates opening of L-type Ca^{2+} channels. This pathway may be responsible for the inhibitory role of SK_{Ca} channels in the PACAP-induced catecholamine secretion in the rat adrenal gland.

Our recent study demonstrated that perfusion with Ca^{2+} free-solution also blunted the PACAP-induced catecholamine output responses almost to the same extent as observed with nifedipine treatment (Fukushima et al., 2001). Therefore, the residual catecholamine output response during the nifedipine treatment may result from Ca^{2+} release from intracellular Ca^{2+} stores. Since apamin does not enhance the nicotinic receptor-mediated adrenal catecholamine secretion that depends on the influx of extracellular Ca^{2+} (Nagayama et al., 2000b; Fukushima et al., 2001), the Ca^{2+} release from intracellular stores during application of PACAP may predominantly contribute to opening SK_{Ca} channels.

In summary, the present study demonstrated that apamin enhanced the PACAP-induced epinephrine and norepinephrine output responses in the isolated perfused rat adrenal gland, and that the enhancement was abolished by treatment with nifedipine. SK_{Ca} channels may play an inhibitory role in the PACAP-induced catecholamine secretion by interfering with L-type Ca^{2+} channels in the rat adrenal gland.

References

Bednarek, M.A., Bugianesi, R.M., Leonard, R.J., Felix, J.P., 1994. Chemical synthesis and structure-function studies of margatoxin, a potent inhibitor of voltage-dependent potassium channel in human T lymphocytes. Biochem. Biophys. Res. Commun. 198, 619–625.

Fukushima, Y., Nagayama, T., Kawashima, H., Hikichi, H., Yoshida, M., Suzuki-Kusaba, M., Hisa, H., Kimura, T., Satoh, S., 2001. Role of calcium channels and adenylate cyclase in the PACAP-induced adrenal catecholamine secretion. Am. J. Physiol. 281, R495–R501 (Regulatory Integrative Comp. Physiol.).

Geng, G., Gaspo, R., Trabelsi, F., Yamaguchi, N., 1997. Role of L-type Ca^{2+} channel in PACAP-induced adrenal catecholamine release in vivo. Am. J. Physiol. 273, R1339–R1345 (Regulatory Integrative Comp. Physiol.).

Ghatei, M.A., Takahashi, K., Suzuki, Y., Gardiner, J., Jones, PM., Bloom, S.R., 1993. Distribution, molecular characterization of pituitary adenylate cyclase-activating polypeptide and its precursor encoding messenger RNA in human and rat tissues. J. Endocrinol. 136, 159–166.

Kawai, T., Watanabe, M., 1986. Blockade of Ca-activated K conductance by apamin in rat sympathetic neurones. Br. J. Pharmacol. 87, 225–232.

Miyata, A., Arimura, A., Dahl, R.R., Minamino, N., Uehara, A., Jiang, L., Culler, M.D., Coy, D.H., 1989. Isolation of a novel 38 residue-hypothalamic polypeptide which stimulates adenylate cyclase in pituitary cells. Biochem. Biophys. Res. Commun. 164, 567–574.

Nagayama, T., Matsumoto, T., Kuwakubo, F., Fukushima, Y., Yoshida, M., Suzuki-Kusaba, M., Hisa, H., Kimura, T., Satoh, S., 1999. Role of calcium channels in catecholamine secretion in the rat adrenal gland. J. Physiol. (London) 520, 503–512.

Nagayama, T., Fukushima, Y., Hikichi, H., Yoshida, M., Suzuki-Kusaba, M., Hisa, H., Kimura, T., Satoh, S., 2000a. Interaction of SK_{Ca} channels and L-type Ca^{2+} channels in catecholamine secretion in the rat adrenal gland. Am. J. Physiol. 279, R1731–R1736 (Regulatory Integrative Comp. Physiol.).

Nagayama, T., Fukushima, Y., Yoshida, M., Suzuki-Kusaba, M., Hisa, H., Kimura, T., Satoh, S., 2000b. Role of potassium channels in catecholamine secretion in the rat adrenal gland. Am. J. Physiol. 279, R448–R454 (Regulatory Integrative Comp. Physiol.).

Neylon, C.B., Lang, R.J., Fu, Y., Bobik, A., Reinhart, P.H., 1999. Molecular cloning and characterization of the intermediate-conductance Ca^{2+}-activated K^+ channel in vascular smooth muscle: relationship between K_{Ca} channel diversity and smooth muscle cell function. Circ. Res. 85, e33–e43.

O'Farrell, M., Marley, P.D., 1997. Multiple calcium channels are required for pituitary adenylate cyclase-activating polypeptide-induced catecholamine secretion from bovine cultured adrenal chromaffin cells. Naunyn-Schmiedeberg's Arch. Pharmacol. 356, 536–542.

Przywara, D.A., Guo, X., Angelilli, M.L., Wakade, T.D., Wakade, A.R., 1996. A non-cholinergic transmitter, pituitary adenylate cyclase-activating polypeptide, utilizes a novel mechanism to evoke catecholamine secretion in rat adrenal chromaffin cells. J. Biol. Chem. 271, 10545–10550.

Stansfeld, C.E., Marsh, S.J., Parcej, D.N., Doly, J.O., Brown, D.A., 1987. Mast cell degranulating peptide and dendrotoxin selectively inhibit a fast-activating potassium current and bind to common neuronal proteins. Neuroscience 23, 893–902.

Tanaka, K., Shibuya, I., Nagamoto, T., Yamashita, H., Kanno, T., 1996. Pituitary adenylate cyclase-activating polypeptide causes rapid Ca^{2+} release from intracellular stores and long lasting Ca^{2+} influx mediated by Na^+ influx-dependent membrane depolarization in bovine adrenal chromaffin cells. Endocrinology 137, 956–966.

Wada, A., Urabe, M., Yuhi, T., Yamamoto, R., Yanagita, T., Niina, H., Kobayashi, H., 1995. Large- and small-conductance Ca^{2+}-activated K^+ channels: their role in the nicotinic receptor-mediated catecholamine secretion in bovine adrenal medulla. Naunyn-Schmiedeberg's Arch. Pharmacol. 352, 545–549.