ELSEVIER

European Journal of Pharmacology 333 (1997) 223–229

ejp

FK506-binding proteins regulate smooth muscle contractility by altering neurotransmitter release

Masaaki Kageyama [a,*], Hiromi Fujita [a], Wakana Goto [a], Katsuhiko Nakata [a], Eiichi Shirasawa [a], Atsushi Kanai [b]

[a] *Ophthalmic Research Division, Santen Pharmaceutical Co. Ltd., 8916-16 Takayama-cho, Ikoma-shi, Nara 630-01, Japan*
[b] *Department of Ophthalmology, Juntendo University School of Medicine, Tokyo 113, Japan*

Received 2 July 1997; accepted 4 July 1997

Abstract

To determine the roles of FK506-binding proteins, receptors for the immunosuppressant FK506, in tachykinin release, we examined the effects of the FK506 derivative ascomycin, [3S-[3R[E(1S,3S,4S)],4S,5R,8S,9E,12R,14R,15S,16R,18S,19S,26aR]]-8-ethyl-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-hexadecahydro-5,19-dihydroxy-3-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylethenyl]-14,16-dimethoxy-4,10,12,18-tetramethyl-15,19-epoxy-3H-pyrido[2,1-c][1,4]oxaazacyclotricosine-1,7,20,21(4H,23H)-tetrone, on the contractility of the rabbit iris sphincter muscle. Ascomycin (10^{-7} to 10^{-5} M) caused concentration-dependent contractions, which were greatly attenuated by preexposure to rapamycin (10^{-5} M), a FK506 receptor antagonist. Similarly, this contractile effect was abolished by preexposure to FK888 (10^{-6} M), a tachykinin receptor antagonist, and to capsaicin (10^{-5} M), a tachykinin-depleting agent. L-type voltage-dependent Ca^{2+} channel blockers, nicardipine (10^{-5} M) and verapamil (5×10^{-5} M), inhibited the ascomycin-induced contraction, but the N-type channel blocker ω-conotoxin (10^{-6} M) did not. These results suggest that ascomycin stimulates tachykinin release by its binding to FK506-binding proteins and the subsequent activation of L-type Ca^{2+} channels. Thus, FK506-binding proteins may regulate muscle contractility by altering transmitter release from peripheral tachykininergic nerves. © 1997 Elsevier Science B.V.

Keywords: Ascomycin; FK506-binding protein; Neurotransmitter release; Smooth muscle contraction; Tachykininergic nerve; Iris sphincter muscle, rabbit

1. Introduction

FK506-binding proteins are cytosolic receptors for the immunosuppressant FK506 (Harding et al., 1989; Siekierka et al., 1989), which is more potent and safer than cyclosporin A (Thomson, 1989). The interaction between FK506 and FK506-binding proteins leads to the inhibition of calcineurin, a serine–threonine protein phosphatase type 2B (Yang et al., 1982), which is a key step in the intracellular signaling pathway responsible for immunosuppression (Clipstone and Crabtree, 1992; O'Keefe et al., 1992). FK506-binding proteins are also distributed in the nervous system (Maki et al., 1990; Steiner et al., 1992) where they are concentrated and colocalized with calcineurin (Steiner et al., 1992). Although this suggests that FK506-binding proteins affect various nervous functions, their roles are not fully understood. Several proteins were identified as calcineurin substrates, such as growth associated protein-43 (Steiner et al., 1992), nitric oxide synthase (Dawson et al., 1993), voltage-dependent Ca^{2+} channels (VDCCs) (Hosey et al., 1986) and dynamin I (Liu et al., 1994), which seem to be involved in neurotransmitter release. Therefore, it was proposed that the regulation of neurotransmitter release might be one of the roles of FK506-binding proteins in the nervous system (Steiner et al., 1992; Snyder and Sabatini, 1995).

Our preliminary study demonstrated that the FK506-derivative ascomycin contracted the rabbit iris sphincter muscle, which is innervated by non-adrenergic, non-cholinergic nerves. These nerves were found to be tachykininergic and involved in the regulation of muscle contractility through the release of substance P-like tachykinins (Leander et al., 1981; Ueda et al., 1982, 1984). Therefore, we hypothesized that ascomycin interacts with tachykininergic nerves and causes contraction. To test this hypothesis, we examined the effects of several inhibitors on the ascomycin-induced contraction in the rabbit iris sphincter muscle. We report here that the contractile effect of ascomycin is due

* Corresponding author. Tel.: (81-7437) 94508; Fax: (81-7437) 94507.

to the stimulation of transmitter release from tachykininergic nerves by interaction with FK506-binding proteins and subsequent activation of L-type VDCCs.

2. Materials and methods

2.1. Tissue preparation and tension measurement

All experiments were conducted in accordance with the Public Health Service Policy and Government Principles Regarding the Care and Use of Animals, and with the Japanese Law on the Protection of Animals. General procedures have previously been described in details (Kageyama et al., 1997a). Briefly, male Japanese White rabbits weighing 2.3 to 3.8 kg were sacrificed by an excess dose of sodium pentobarbital and strips of rabbit iris sphincter muscle (1 mm in width and 5 mm in length) were prepared. Each strip was suspended between two hooks and placed in an organ bath containing Krebs–Henseleit solution (KHS), which was maintained at 37°C and gassed with 95% O_2 and 5% CO_2. Tension was measured at a resting tension of 0.2 mN with a force displacement transducer (TB-612T, Nihon Kohden, Tokyo, Japan), which was connected to one of the hooks. Changes in tension were recorded on a thermal pen recorder (T-685G, Nihon Kohden). At least 60 min was allowed for equilibration before each protocol was started. In each experiment, muscle viability was verified by transient exposure to KHS containing 45.9 mM KCl. One strip obtained from one eye of each animal was exposed to ascomycin, and served as control. The other strip obtained from the contralateral eye of the same animal was preexposed to inhibitors or antagonists before ascomycin application. Ascomycin was cumulatively applied to each strip only once. Contractions induced by ascomycin are expressed as a percentage of those induced by 45.9 mM KCl.

2.2. Drugs

KHS contained (in mM): NaCl, 118.4; KCl, 4.7; $CaCl_2$, 2.4; $NaHCO_3$, 25.0; $MgCl_2$, 1.2; KH_2PO_4, 1.2 and glucose, 11.7 (pH 7.4). Potassium concentration was elevated by adding an appropriate aliquot of 2 M KCl. The following pharmacological agents were used: ascomycin, [3S-[3R[E(1S,3S,4S)],4S,5R,8S,9E,12R,14R,15S,16R,18S,19S,26aR]]-8-ethyl-5,6,8,11,12,13,14,15,16,17,18,19,24,25,26,26a-hexadecahydro-5,19-dihydroxy-3-[2-(4-hydroxy-3-methoxy-cyclohexyl)-1-methylethenyl]-14,16-dimethoxy-4,10,12,18-tetramethyl-15,19-epoxy-3H-pyrido-[2,1-c][1,4]oxaazacyclotricosine-1,7,20,21(4H,23H)-tetrone and rapamycin, obtained from Calbiochem (La Jolla, CA, USA), spantide, substance P and ω-conotoxin, from Peptide Institute (Osaka, Japan), FK888, from Wako (Osaka, Japan) and carbachol, capsaicin, verapamil and nicardipine, from Sigma (St. Louis, MO, USA). Ascomycin, rapamycin and capsaicin were dissolved in 100% ethanol and FK888 and nicardipine in dimethyl sulfoxide (DMSO). The other chemicals were dissolved in distilled water. The final concentrations of ethanol and DMSO were kept at less than 0.325 and 0.1%, respectively.

2.3. Statistical analysis of data

Each value represents the mean ± S.E.M. The statistical significance was determined by the Student's *t*-test for unpaired data. Differences were assumed to be significant when $P < 0.05$.

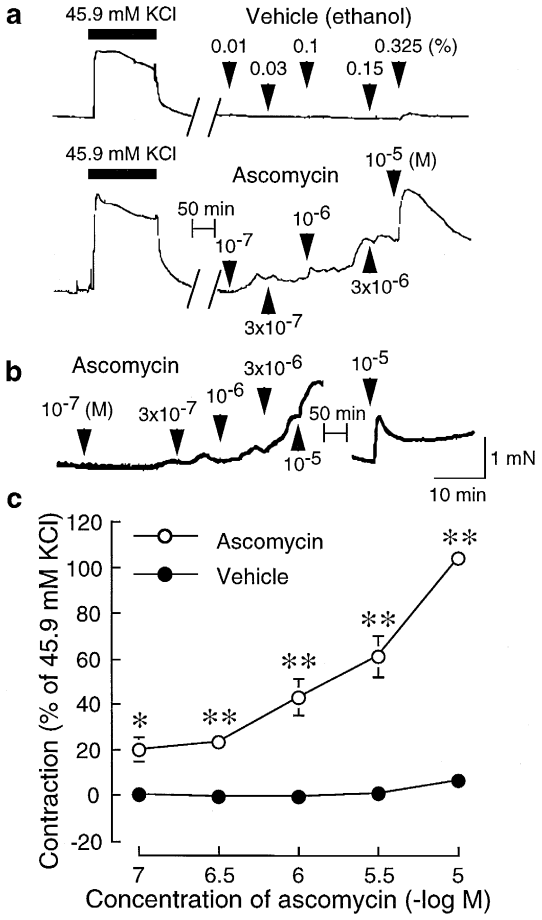

Fig. 1. Contractile effects of ascomycin and its vehicle on isolated rabbit iris sphincter muscle. Representative recordings of the effects of ascomycin and vehicle (a); repeated application of ascomycin (b); concentration–response curves for the contractile effects of ascomycin and vehicle (c). In (a) and (c), one of the two strips from each animal was exposed to ascomycin (○) and the other to vehicle corresponding to ascomycin solutions (●). In (b), ascomycin was cumulatively applied and washed out after application of 10^{-5} M. Then, ascomycin at 10^{-5} M was repeatedly applied to the same preparation. The ensuing contraction was expressed as a percentage of the contraction induced by 45.9 mM KCl just before application of rapamycin. Each value represents the mean ± S.E.M. of 5 different preparations. * $P < 0.05$; ** $P < 0.01$, compared with preparations exposed to vehicle.

3. Results

3.1. Effects of ascomycin on tension

As shown in Fig. 1a, the application of 45.9 mM KCl resulted in a rapid onset of contraction. In contrast, ascomycin caused a slow onset and long-lasting contraction, which was concentration-dependent from 10^{-7} to 10^{-5} M (Fig. 1a and c). Following the application of ascomycin, the tension gradually increased, reached a maximum within 5 to 10 min depending on concentrations used and then decreased towards baselines. The contraction induced by the second application of ascomycin (10^{-5} M) was less than that by the first application, suggesting that it causes tachyphylaxis (Fig. 1b). The maximum contractile response to ascomycin, which would be obtained at more than 10^{-5} M, was not examined, because of the poor water solubility of ascomycin. The vehicle (ethanol) at corresponding concentrations with ascomycin solutions had only a small contractile effect, which was significantly different from that of ascomycin at any concentrations (Fig. 1a and c).

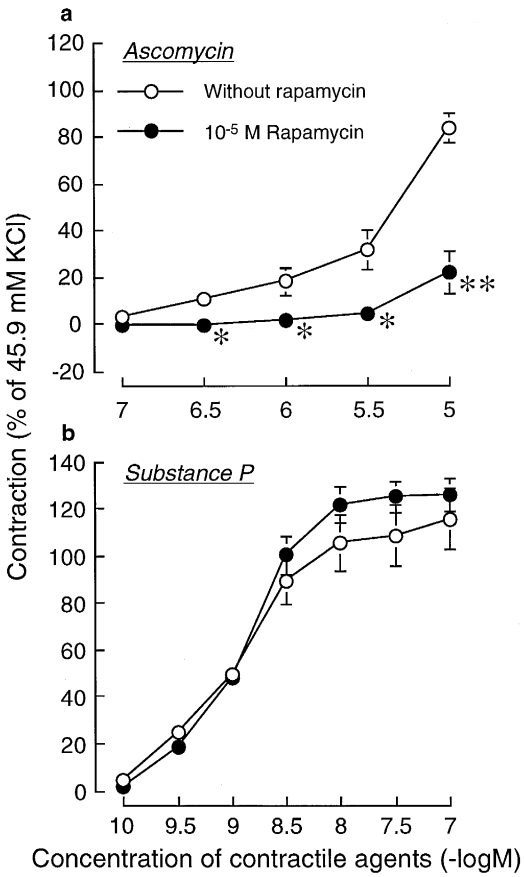

Fig. 2. Concentration–response curves for the contractile effect of ascomycin (a) and substance P (b) in the absence (○) or presence (●) of the FK506 receptor antagonist rapamycin. One of the two strips from each animal was exposed to rapamycin (10^{-5} M) for 30 min, and the other served as control. The contractile agent under study was then applied cumulatively (during continuing exposure to rapamycin in one strip). The ensuing contraction was expressed as a percentage of the contraction induced by 45.9 mM KCl just before application of rapamycin Each value represents the mean ± S.E.M. of 5 to 7 different preparations. * $P < 0.05$; ** $P < 0.01$, compared with preparations without rapamycin.

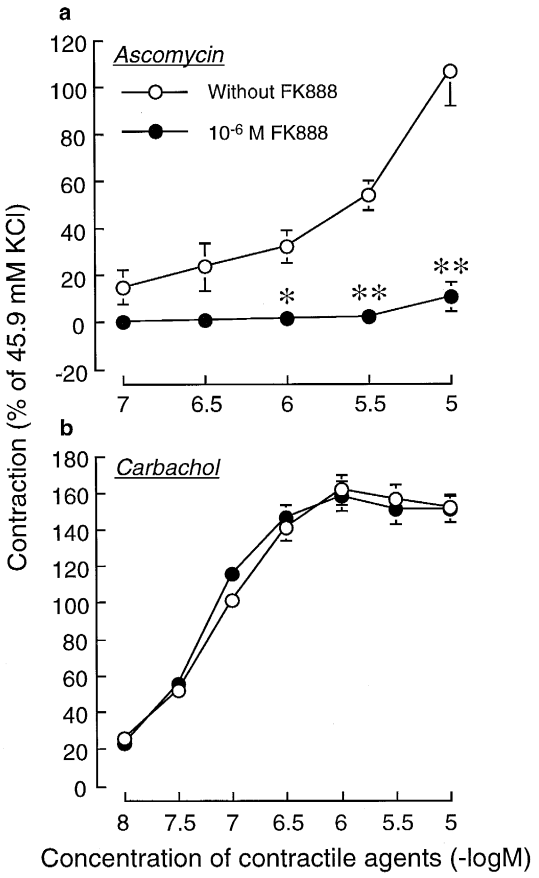

Fig. 3. Effects of FK888, a substance P receptor antagonist, on ascomycin- (a) and carbachol-induced contractions (b). One of the two strips from each animal was exposed to FK888 (10^{-6} M) for 30 min (●), and the other served as control (○). The contractile agent under study was then applied cumulatively (during continuing exposure to rapamycin in one strip). The ensuing contraction was expressed as a percentage of the contraction induced by 45.9 mM KCl just before application of FK888. Each value represents the mean ± S.E.M. of 4 to 5 different preparations. * $P < 0.05$; ** $P < 0.01$, compared with preparations without FK888.

3.2. Effects of rapamycin on ascomycin-induced contraction

To determine the involvement of FK506-binding proteins in the contractile effect of ascomycin, we examined the effects of rapamycin on ascomycin-induced contraction. Since, like FK506, rapamycin binds to FK506-binding proteins, it is used as an antagonist for FK506-binding proteins (Dumont et al., 1990; O'Keefe et al., 1992). As

shown in Fig. 2, a 30 min preexposure to rapamycin (10^{-5} M) significantly inhibited the contractile effect of ascomycin, but it did not affect the contraction induced by substance P (10^{-10} to 10^{-7} M). Rapamycin itself did not have any contractile effect (data not shown).

3.3. Effect of FK888 and capsaicin on ascomycin-induced contraction

To determine whether the ascomycin-induced contraction was due to neurotransmitter release from tachykininergic nerves, we examined the effects of FK888 and capsaicin on the ascomycin-induced contraction. FK888 is a newly developed substance P receptor antagonist (Fujii et al., 1992). Capsaicin is a pungent ingredient in red peppers, which depletes neurotransmitters from tachykininergic nerve endings (Holzer, 1991). As shown in Fig. 3, the contractile effect of ascomycin was significantly inhibited by a 30 min preexposure to FK888 (10^{-6} M), which also inhibited the substance P-induced contraction (data not shown), but not that by carbachol (10^{-8} to 10^{-5} M). A similar result was obtained with another substance P receptor antagonist, spantide (data not shown). Capsaicin (10^{-5} M) caused a transient contraction and a subsequent application did not cause contraction (data not shown), suggesting that tachykininergic nerves were already chemically denervated. Preincubation with capsaicin for 60 min abolished the ascomycin-induced contraction, whereas it did not affect that induced by substance P (Fig. 4).

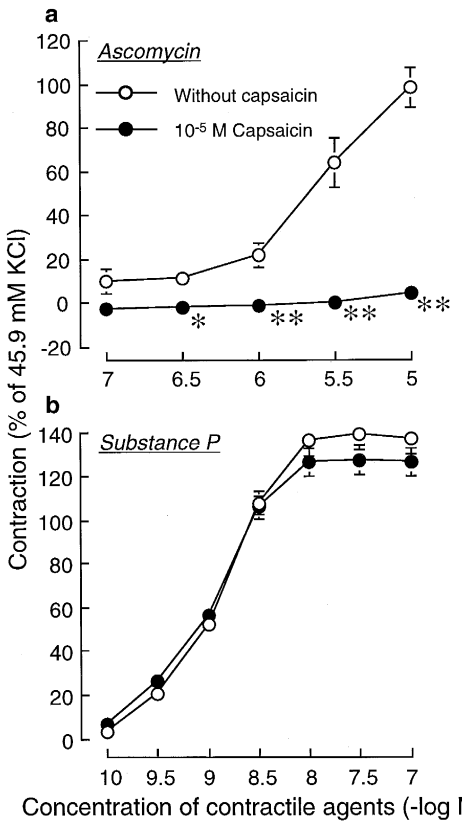

Fig. 4. Effects of capsaicin, a substance P-depleting agent, on ascomycin- (a) and substance P-induced contractions (b). One of the two strips from each animal was exposed to capsaicin (10^{-5} M) for 60 min (●) and the other served as control (○). The contractile agent under study was then applied cumulatively (during continuing exposure to rapamycin in one strip). The ensuing contraction was expressed as a percentage of the contraction induced by 45.9 mM KCl just before application of capsaicin. Each value represents the mean ± S.E.M. of 6 to 7 different preparations. * $P < 0.05$; ** $P < 0.01$, compared with preparations without capsaicin.

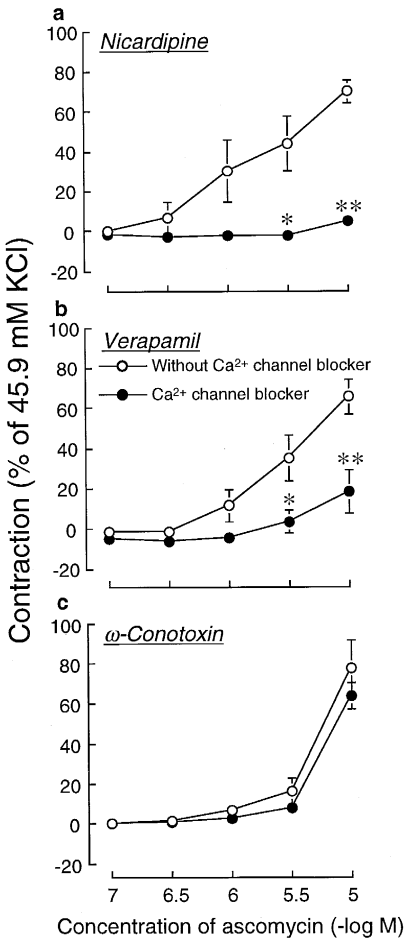

Fig. 5. Effects of nicardipine (a), verapamil (b), L-type voltage-dependent Ca^{2+} channel blockers, and ω-conotoxin (c), an N-type blocker, on ascomycin-induced contractions. One of the two strips from each animal was exposed to nicardipine (10^{-5} M), verapamil (5×10^{-5} M) or ω-conotoxin (10^{-6} M) for 30 min (●), and the other served as control (○). Ascomycin was then applied cumulatively (during continuing exposure to Ca^{2+} channel blockers in one strip). The ensuing contraction was expressed as a percentage of the contraction induced by 45.9 mM KCl just before application of each Ca^{2+} channel blocker. Each value represents the mean ± S.E.M. of 5 to 6 different preparations. * $P < 0.05$; ** $P < 0.01$, compared with preparations without each blocker.

3.4. Effects of Ca^{2+} channel blockers on ascomycin-induced contraction

To obtain further insight into the mechanism of ascomycin-induced contraction, we examined the effects of

nicardipine and verapamil, L-type VDCC blockers and ω-conotoxin, an N-type blocker, on the ascomycin-induced contraction. As shown in Fig. 5a, when nicardipine (10^{-5} M) was applied for 30 min before the ascomycin addition, it greatly attenuated the ascomycin-induced contraction. However, nicardipine had no effect on substance P-induced contraction. The concentrations producing 50% of the contractile response to substance P were 9.33 ± 0.09 and 9.40 ± 0.04 ($-\log M$) in the absence and presence of nicardipine, respectively ($n = 5$, not significant). Using the same protocol, verapamil (5×10^{-5} M) was also effective in inhibiting ascomycin-induced contraction (Fig. 5b). In contrast, the N-type VDCC blocker ω-conotoxin (10^{-6} M) did not affect the ascomycin-induced contraction (Fig. 5c).

4. Discussion

The main finding of the present study is that the rabbit iris sphincter muscle contracted in response to ascomycin, which is an immunosuppressant with a chemical structure similar to FK506 and acts through the same mechanisms of action as FK506 (Petros et al., 1991; Dumont et al., 1992). The contractile effect of ascomycin was greatly attenuated by preexposure to FK888 (Fujii et al., 1992) and spantide, substance P receptor antagonists (Folkers et al., 1984). This suggests that ascomycin causes contraction through activation of tachykinin receptors. The contractile effect of ascomycin was also inhibited by capsaicin, an agent that depletes substance P-like tachykinins from tachykininergic nerves (Holzer, 1991). Capsaicin had no effect on the substance P-induced contraction, suggesting that it inhibits function of tachykininergic nerve, but does not directly affect muscle function or tachykinin receptors. Thus, besides the immunosuppressive effect, ascomycin may have a stimulatory effect on neurotransmitter release from peripheral tachykininergic nerves in the iris sphincter muscle.

Interaction between ascomycin or FK506 and FK506-binding proteins, their cytosolic receptors (Harding et al., 1989; Siekierka et al., 1989), is a key event in the signaling pathway leading to immunosuppression (Liu et al., 1991). Interestingly, we found that the contractile effect of ascomycin was significantly inhibited by rapamycin, which is used as an antagonist for FK506-binding proteins (Dumont et al., 1990; O'Keefe et al., 1992), because the complex of rapamycin and FK506-binding proteins couples to different signaling pathways from that of FK506 or ascomycin and FK506-binding proteins (Price et al., 1992; Terada et al., 1993). Rapamycin did not affect the substance P-induced contraction, suggesting that it does not interact with tachykinin receptors or subsequent signal transduction, but specifically antagonizes FK506-binding proteins. Therefore, the complex of ascomycin and FK506-binding proteins seems to mediate its stimulatory effect on neurotransmitter release from tachykininergic nerves as well as immunosuppression.

In the present study, rapamycin inhibited the contractile effect of ascomycin, but it did not cause any contraction per se. We previously demonstrated that cyclosporin A had the same stimulatory effect on tachykinin release as that of ascomycin (Kageyama et al., 1997b). The complex of FK506-binding proteins with ascomycin inhibits the activity of the protein phosphatase calcineurin (Clipstone and Crabtree, 1992; O'Keefe et al., 1992), whereas that with rapamycin does not affect it, but instead inhibits S6 kinase (Price et al., 1992; Terada et al., 1993). Cyclosporin A binds to different immunophilins, so-called cyclophilins, and the complex of cyclosporin A and cyclophilins inhibits calcineurin activity as seen with that of FK506 and FK506-binding proteins (Clipstone and Crabtree, 1992; O'Keefe et al., 1992). These similar properties of ascomycin and cyclosporin A suggest that calcineurin inhibition is involved in the stimulatory effect of ascomycin on tachykinin release.

This view is supported by an earlier study demonstrating that the potency of calcineurin inhibition by several FK506 derivatives correlates with that of their stimulatory effects on sympathetic nerve activity (Lyson et al., 1993). Calcineurin inhibition also mediated stimulation by FK506 of glutaminergic neurotransmission in rat cortical neurons (Victor et al., 1995). On the other hand, calcineurin inhibition by FK506 suppressed dopamine and acetylcholine release from a rat pheochromocytoma cell line, PC12 (Snyder and Sabatini, 1995). These opposite effects of FK506-binding protein ligands on neurotransmitter release may be due to different signaling pathways coupled to FK506-binding proteins and calcineurin, because substrates for calcineurin are diverse (Hosey et al., 1986; Steiner et al., 1992; Dawson et al., 1993; Liu et al., 1994).

Recently, we demonstrated that L- and N-type, but not P-type, VDCCs regulate neurotransmitter release from tachykininergic nerves in the rabbit iris sphincter muscle (Kageyama et al., 1997a). In the present study, nicardipine and verapamil, L-type VDCC blockers, greatly inhibited the ascomycin-induced contraction, but nicardipine did not affect the substance P-induced one. This suggests that nicardipine and probably verapamil presynaptically act to inhibit neurotransmitter release from tachykininergic nerves. In contrast, the N-type VDCC blocker ω-conotoxin had no effect on the ascomycin-induced contraction. Thus, ascomycin may stimulate transmitter release from tachykininergic nerves predominantly by activating L-type VDCCs located on nerve endings, even if both L- and N-type VDCCs regulate it (Kageyama et al., 1997a).

Predominant activation by ascomycin of L-type VDCCs may be due to differences in the regulation of L- and N-type VDCC function by protein kinases and phosphatases. Protein kinase A phosphorylates both L- and N-type VDCCs (Hell et al., 1995) and subsequently activates L-type, but inactivates N-type VDCCs (Sculptoreanu

et al., 1995). In contrast, calcineurin dephosphorylates and inactivates L-type VDCCs (Armstrong, 1989), whereas it probably activates N-type VDCCs, although it is unknown if N-type VDCCs are a calcineurin substrate. Thus, calcineurin inhibition by ascomycin may result in predominant activation of L-type VDCCs. Alternatively, N-type VDCCs may not be a calcineurin substrate. If so, calcineurin inhibition by ascomycin may phosphorylate and activate L-type VDCCs, but may not affect N-type VDCCs. Further studies are needed to clarify the exact mechanisms for predominant activation of L-type VDCCs by ascomycin.

In conclusion, we found that ascomycin caused FK888- and capsaicin-sensitive contraction in the isolated rabbit iris sphincter muscle, which was inhibited by rapamycin and L-type VDCC blockers. These results suggest that ascomycin stimulates tachykinin release by interacting with FK506-binding proteins and by subsequent activation of L-type VDCCs. Thus, FK506-binding proteins may regulate iris sphincter muscle contractility by altering tachykinin release.

Acknowledgements

We are grateful to Dr. Shiro Mita and Dr. Peter Reinach for helpful discussions.

References

Armstrong, D.L., 1989. Calcium channel regulation by calcineurin, a Ca^{2+}-activated phosphatase in mammalian brain. Trends Neurosci. 12, 117.

Clipstone, N.A., Crabtree, G.R., 1992. Identification of calcineurin as a key signalling enzyme in T-lymphocyte activation. Nature 357, 695.

Dawson, T.M., Steiner, J.P., Dawson, V.L., Dinerman, J.L., Uhl, G.R., Snyder, S.H., 1993. Immunosuppressant FK506 enhances phosphorylation of nitric oxide synthase and protects against glutamate neurotoxicity. Proc. Natl. Acad. Sci. USA 90, 9808.

Dumont, F.J., Melino, M.R., Staruch, M.J., Koprak, S.L., Fischer, P.A., Sigal, N.H., 1990. The immunosuppressive macrolides FK-506 and rapamycin act as reciprocal antagonists in murine T-cells. J. Immunol. 144, 1418.

Dumont, F.J., Staruch, M.J., Koprak, S.L., Siekierka, J.J., Lin, C.S., Harrison, R., Sewell, T., Kindt, V.M., Beattie, T.R., Wyvratt, M., Sigal, N.H., 1992. The Immunosuppressive and toxic effects of FK-506 are mechanistically related: Pharmacology of a novel antagonist of FK-506 and rapamycin. J. Exp. Med. 176, 751.

Folkers, K., Håkanson, R., Hörig, J., Jie-Cheng, X., Leander, S., 1984. Biological evaluation of substance P antagonists. Br. J. Pharmacol. 83, 449.

Fujii, T., Murai, M., Morimoto, H., Maeda, Y., Yamaoka, M., Hagiwara, D., Miyake, H., Ikari, N., Matsuo, M., 1992. Pharmacological profile of a high affinity dipeptide NK1 receptor antagonist, FK888. Br. J. Pharmacol. 107, 785.

Harding, M.W., Galat, A., Uehling, D.E., Schreiber, S.L., 1989. A receptor for the immunosuppressant FK506 is a *cis–trans* peptidyl-prolyl isomerase. Nature 341, 758.

Hell, J.W., Yokoyama, C.T., Breeze, L.J., Chavkin, C., Catterall, W.A., 1995. Phosphorylation of presynaptic and postsynaptic calcium channels by cAMP-dependent protein kinase in hippocampal neurons. EMBO J. 14, 3036.

Holzer, P., 1991. Capsaicin: Cellular targets, mechanisms of action, and selectivity for thin sensory neurons. Pharmacol. Rev. 43, 143.

Hosey, M.M., Borsotto, M., Lazdunski, M., 1986. Phosphorylation and dephosphorylation of dihydropyridine-sensitive voltage-dependent Ca^{2+} channel in skeletal muscle membranes by cAMP- and Ca^{2+}-dependent processes. Proc. Natl. Acad. Sci. USA 83, 3733.

Kageyama, M., Fujita, H., Nakata, K., Shirasawa, E., 1997a. Involvement of both L-type and N-type voltage-dependent Ca^{2+} channels in KCl- and veratridine-evoked transmitter release from non-adrenergic, non-cholinergic nerves in the rabbit iris sphincter muscle. Naunyn-Schmiedebergs Arch, Pharmacol. 355, in press.

Kageyama, M., Fujita, H., Nakata, K., Shirasawa, E., Kanai, A., 1997b. Effects of the immunosuppressant cyclosporin A on neurotransmitter release from peripheral non-adrenergic, non-cholinergic nerves. Naunyn-Schmiedebergs Arch. Pharmacol., in press.

Leander, S., Håkanson, R., Rosell, S., Folkers, K., Sundler, F., Tornqvist, K., 1981. A specific substance P antagonist blocks smooth muscle contractions induced by non-cholinergic, non-adrenergic nerve stimulation. Nature 294, 467.

Liu, J., Farmer, J.D. Jr., Lane, W.S., Friedman, J., Weissman, I., Schreiber, S.L., 1991. Calcineurin is a common target of cyclophilin-cyclosporin A and FKBP–FK506 complexes. Cell 66, 807.

Liu, J.-P., Sim, A.T.R., Robinson, P.J., 1994. Calcineurin inhibition of dynamin I GTPase activity coupled to nerve terminal depolarization. Science 265, 970.

Lyson, T., Ermel, L.D., Belshaw, P.J., Alberg, D.G., Schreiber, S.L., Victor, R.G., 1993. Cyclosporine- and FK506-induced sympathetic activation correlates with calcineurin-mediated inhibition of T-cell signaling. Circ. Res. 73, 596.

Maki, N., Sekiguchi, F., Nishimaki, J., Miwa, K., Hayano, T., Takahashi, N., Suzuki, M., 1990. Complementary DNA encoding the human T-cell FK506-binding protein, a peptidylprolyl *cis–trans* isomerase distinct from cyclophilin. Proc. Natl. Acad. Sci. USA 87, 5440.

O'Keefe, S.J., Tamura, J., Kincaid, R.L., Tocci, M.J., O'Neill, E.A., 1992. FK-506- and CsA-sensitive activation of the interleukin-2 promoter by calcineurin. Nature 357, 692.

Petros, A.M., Gampe, R.T. Jr., Gemmecker, G., Neri, P., Holzman, T.F., Edalji, R., Hochlowski, J., Jackson, M., McAlpine, J., Luly, J.R., Pilot-Matias, T., Pratt, S., Fesik, S.W., 1991. NMR studies of an FK-506 analogue, [U-^{13}C]ascomycin, bound to FKBP: Conformation and regions of ascomycin involved in binding. J. Med. Chem. 34, 2925.

Price, D.J., Grove, J.R., Calvo, V., Avruch, J., Bierer, B.E., 1992. Rapamycin-induced inhibition of the 70 kDa S6 protein kinase. Science 257, 973.

Sculptoreanu, A., Figourov, A., De Groat, W.C., 1995. Voltage-dependent potentiation of neuronal L-type calcium channels due to state-dependent phosphorylation. Am. J. Physiol. 269, C725.

Siekierka, J.J., Hung, S.H.Y., Poe, M., Lin, C.S., Sigal, N.H., 1989. A cytosolic binding protein for the immunosuppressant FK506 has peptidyl-prolyl isomerase activity but is distinct from cyclophilin. Nature 341, 755.

Snyder, S.H., Sabatini, D.M., 1995. Immunophilins and the nervous system. Nature Med. 1, 32.

Steiner, J.P., Dawson, T.M., Fotuhi, M., Glatt, C.E., Snowman, A.M., Cohen, N., Snyder, S.H., 1992. High brain densities of the immunophilin FKBP colocalized with calcineurin. Nature 358, 584.

Terada, N., Franklin, R.A., Lucas, J.J., Blenis, J., Gelfand, E.W., 1993. Failure of rapamycin to block proliferation once resting cells have entered the cell cycle despite inactivation of p70 S6 kinase. J. Biol. Chem. 268, 12062.

Thomson, A.W., 1989. FK-506: How much potential. Immunol. Today 10, 6.

Ueda, N., Muramatsu, I., Fujiwara, M., 1984. Capsaicin and bradykinin-

induced substance P-ergic responses in the iris sphincter muscle of the rabbit. J. Pharmacol. Exp. Ther. 230, 469.

Ueda, N., Muramatsu, I., Hayashi, H., Fujiwara, M., 1982. Trigeminal nerve: The possible origin of substance P-nergic response in isolated rabbit iris sphincter muscle. Life Sci. 31, 369.

Victor, R.G., Thomas, G.D., Marban, E., O'Rourke, B., 1995. Presynaptic modulation of cortical synaptic activity by calcineurin. Proc. Natl. Acad. Sci. USA 92, 6269.

Yang, S.-D., Tallant, E.A., Cheung, W.Y., 1982. Calcineurin is a calmodulin-dependent protein phosphatase. Biochem. Biophys. Res. Commun. 106, 1419.