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The diacylglycerol lipase inhibitor RHC-80267 potentiates the relaxation to acetylcholine in rat mesenteric artery by anti-cholinesterase action

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Abstract

The diacylglycerol lipase inhibitor 1,6-bis(cyclohexyloximinocarbonylamino) hexane (RHC-80267) was tested for its effect on acetylcholine-evoked relaxation in rat mesenteric artery. In artery contracted with either noradrenaline or KCl, RHC-80267 (0.1–10 μM) potentiated the relaxation evoked by acetylcholine. The effect of RHC-80267 was not affected by nitric oxide synthase inhibition or by inhibitors of protein kinase C or of phospholipase A_2. The diacylglycerol analogue 1-oleoyl-2-acetyl-*sn*-glycerol did not change the relaxation to acetylcholine. RHC-80267 did not affect the relaxation evoked by carbachol, by the nitric oxide donor SNAP (*S*-nitroso-*N*-acetylpenicillamine) or by the K^+ channel opener cromakalim. Neostigmine, a cholinesterase inhibitor, produced the same effect as RHC-80267 on acetylcholine-evoked relaxation. When tested on cholinesterase in brain homogenate, RHC-80267 concentration-dependently inhibited cholinesterase activity with an IC_{50} of 4 μM. These results indicate that the potentiation of acetylcholine-evoked responses by RHC-80267 in rat mesenteric artery is caused by the inhibition of the cholinesterase activity in the vascular wall.

Keywords: Endothelium-dependent relaxation; Acetylcholine; RHC-80267; Cholinesterase; Diacylglycerol lipase; Mesenteric artery

1. Introduction

The endothelium-dependent relaxation evoked by acetylcholine in pre-contracted arteries has been reported to be mediated by several factors including nitric oxide (NO) (Palmer et al., 1987), prostacyclin (Moncada and Vane, 1978) and an endothelium-derived hyperpolarizing factor (EDHF). The EDHF-dependent relaxation is resistant to nitric oxide synthase and cyclo-oxygenase inhibitors (Feletou and Vanhoutte, 1988; Taylor and Weston, 1988). The chemical nature and the mechanism of action of EDHF remain elusive, although several models have been proposed. Among the hypotheses, arachidonic acid derivatives have been reported to play an important role in EDHF-mediated responses (Fleming, 2004). One source of arachidonic acid is the hydrolysis of diacylglycerol by a diacylglycerol lipase. The aim of the present study was to investigate whether this pathway could be involved in the EDHF-dependent relaxation evoked by acetylcholine in rat mesenteric artery, by using RHC-80267. This compound has been reported to be a selective inhibitor of diacylglycerol lipase (Konrad et al., 1994) and has been widely used to investigate the role played by diacylglycerol or arachidonic acid (Mason-Garcia et al., 1992; Nishimaru et al., 2003; Shlykov and Sanborn, 2004; Suzuki et al., 2002; Venkatachalam et al., 2003). Results indicated that at micromolar concentration RHC-80267 potentiated the relaxation to acetylcholine and that this effect was not related to the inhibition of diacylglycerol lipase but resulted from the inhibition of cholinesterase activity.

2. Materials and methods

2.1. Mesenteric artery contraction

Normotensive Wistar–Kyoto (WKY) male rats of two origins were used: WKY/Nico (Charles River, Bruxelles, Belgium) and

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WKY/Kyo@Rj (Janvier, Le Genest St Isle, France). All protocols were in accordance with institutional guidelines for the use of experimental animals. Rats were anaesthetised with diethyl ether and killed by decapitation at 14 weeks at a body weight of 320–350 g. The superior mesenteric artery was rapidly removed and immersed in physiological solution (composition in mM: NaCl 122, KCl 5.9, $NaHCO_3$ 15, glucose 11, $MgCl_2$ 1.25 and $CaCl_2$ 1.25) gassed with a mixture of 95% O_2–5% CO_2. Indomethacin (10 μM) was added to all solutions. The artery was carefully cleaned of all fat and connective tissue. A segment, about 2 mm in length, was mounted in a wire myograph (Model 500A, Danish Myo Technology A/S, Aarhus, Denmark) as described (Ghisdal et al., 1999). The vessel was set at a tension equivalent to that generated at 0.9 times the diameter of the vessel at 100 mm Hg. After an equilibration period, each preparation was contracted by changing the physiological solution in the bath to a depolarizing 100 mM KCl solution (composition in mM: NaCl, 27; KCl, 100; $NaHCO_3$, 15; $MgCl_2$, 1.25; $CaCl_2$, 1.25; and glucose, 11). Endothelium integrity was tested by measuring the relaxation evoked by acetylcholine (1 μM). After preincubation with or without inhibitors for 30 min, contraction was evoked either by the 100 mM KCl solution, when NO-evoked relaxation was tested, or by adding noradrenaline (0.5 μM) in the bath, when EDHF type relaxation or relaxation to the K^+ channel opener cromakalim was tested. KCl-evoked contraction was performed in the presence of phentolamine (1 μM) to rule out the contribution of α-adrenergic transmitter released by nerve endings. Except when acetylcholine-evoked NO-dependent relaxation was tested, the NO synthase inhibitor *N*ω-nitro-L-arginine (L-NOArg, 100 μM) was added in the physiological solution. Relaxations were evoked by cumulative addition of the relaxing agent into the bath when contraction had reached a plateau.

2.2. In vitro cholinesterase activity

Rat brain homogenate was used for determining cholinesterase activity by a modification of the Ellman's method (Bores et al., 1996). Rat brain homogenate (50 mg tissue in 1 ml of 0.05 M phosphate buffer, pH 7.2) was preincubated without or with different concentrations of RHC-80267 or of neostigmine for 10 min at 37 °C in 0.05 M phosphate buffer pH 7.2 containing 0.25 mM DTNB (dithionitrobenzoate, Ellman's reagent). Acetylthiocholine (0.3 mM) was then quickly added and the change in absorbance was monitored at 412 nm in a spectrophotometer (Perkin-Elmer). Percent inhibition of the cholinesterase activity was determined by comparison of the Δ absorbance/minute values measured in the absence (control value) or in the presence of inhibitor.

2.3. Statistical analysis

Results were expressed as mean ± S.E.M. Comparisons were made by analysis of variance (ANOVA) followed by Bonferroni test; $P<0.05$ being considered significant. pD_2 values of acetylcholine ($-\log EC_{50}$, concentration producing half-maximal effect) were obtained by non-linear regression of the individual concentration–response curves and presented as means ± S.E.M. pIC_{50} values ($-\log IC_{50}$, concentration producing 50% inhibition of the activity) were calculated by non-linear regression of the mean data (Prism, GraphPad).

2.4. Drugs

RHC-80267 (1,6-bis(cyclohexyloximinocarbonylamino)-hexane), Ro-31-8220 (1-[3-(amidinothio)propyl-1*H*-indol-3-yl]-3-(1-methyl-1*H*-indol-3-yl)maleimide methane sulfonate) and 1-oleoyl-2-acetyl-*sn*-glycerol were purchased from Calbiochem. Calphostin C was from Alexis. Cromakalim and OBAA (4-(4-octadecylphenyl)-4-oxobutenoic acid) were from Tocris. Neostigmine methyl sulfate was from ICN. All other compounds were obtained from Sigma. RHC-80267 and SNAP (*S*-nitroso-*N*-acetylpenicillamine) were dissolved in ethanol. Ro-31-8220, 1-oleoyl-2-acetyl-*sn*-glycerol, calphostin C and cromakalim were dissolved in DMSO. Stock solution of indomethacin was prepared in 2% Na_2CO_3.

3. Results

3.1. Effect of RHC-80267 on the EDHF type relaxation in mesenteric artery

The EDHF type relaxation evoked by acetylcholine was measured in noradrenaline-contracted arteries in the presence of L-NOArg and indomethacin (Ghisdal et al., 1999). Acetylcholine was more potent to evoke EDHF type relaxation in WKY/Nico (pD_2 6.41 ± 0.06, $n=33$) than in WKY/Kyo@Rj (pD_2 5.87 ± 0.18, $n=6$, $P<0.01$ versus WKY/Nico). In both types of rats, preincubation of artery rings with RHC-80267 produced a concentration-dependent leftward shift of the concentration–effect curve to acetylcholine (Fig. 1A, Table 1). In the presence of 10 μM RHC-80267, pD_2 values of acetylcholine increased to 7.22 ± 0.07 in WKY/Nico ($n=6$, $P<0.01$ compared to control) and 7.20 ± 0.17 in WKY/Kyo@Rj ($n=5$, $P<0.01$ compared to control). The amplitude of the contraction evoked by noradrenaline was slightly decreased in the presence of RHC-80267, but this difference did not reach a statistically significant level (Table 1).

Inhibition of diacylglycerol lipase by RHC-80267 results in the accumulation of diacylglycerol, which is known to activate protein kinase C. In order to investigate the involvement of protein kinase C in the potentiation of the relaxation to acetylcholine by RHC-80267, relaxation to acetylcholine was performed in the presence and absence of protein kinase C inhibitors calphostin C (1 μM) or Ro-31-8220 (1 μM) with or without RHC-80267 (10 μM). Preincubation with calphostin C and with calphostin C plus RHC-80267 decreased the contraction evoked by noradrenaline by 35 ± 10% ($n=4$, $P<0.05$) and 50 ± 9% ($n=4$, $P<0.05$), respectively, whereas the other drugs did not significantly affect the pre-constriction level (Table 1). The analogue of diacylglycerol, 1-oleoyl-2-acetyl-*sn*-glycerol (80 μM) was used to determine whether diacylglycerol could be directly involved in the EDHF type relaxation evoked by acetylcholine. Results indicated that the potentiation of acetylcholine-evoked relaxation by RHC-80267 was not affected by the protein kinase C inhibitors and that 1-oleoyl-2-acetyl-*sn*-glycerol did not change the relaxation evoked by acetylcholine (Table 1). Inhibition of diacylglycerol lipase also affects the production of arachidonic acid from diacylglycerol. Since arachidonic acid is also produced by phospholipase A_2, the concentration–relaxation curve to acetylcholine was measured in arteries preincubated in the presence or in the absence of the phospholipase A_2 inhibitor OBAA (5 μM). OBAA did not change the concentration–

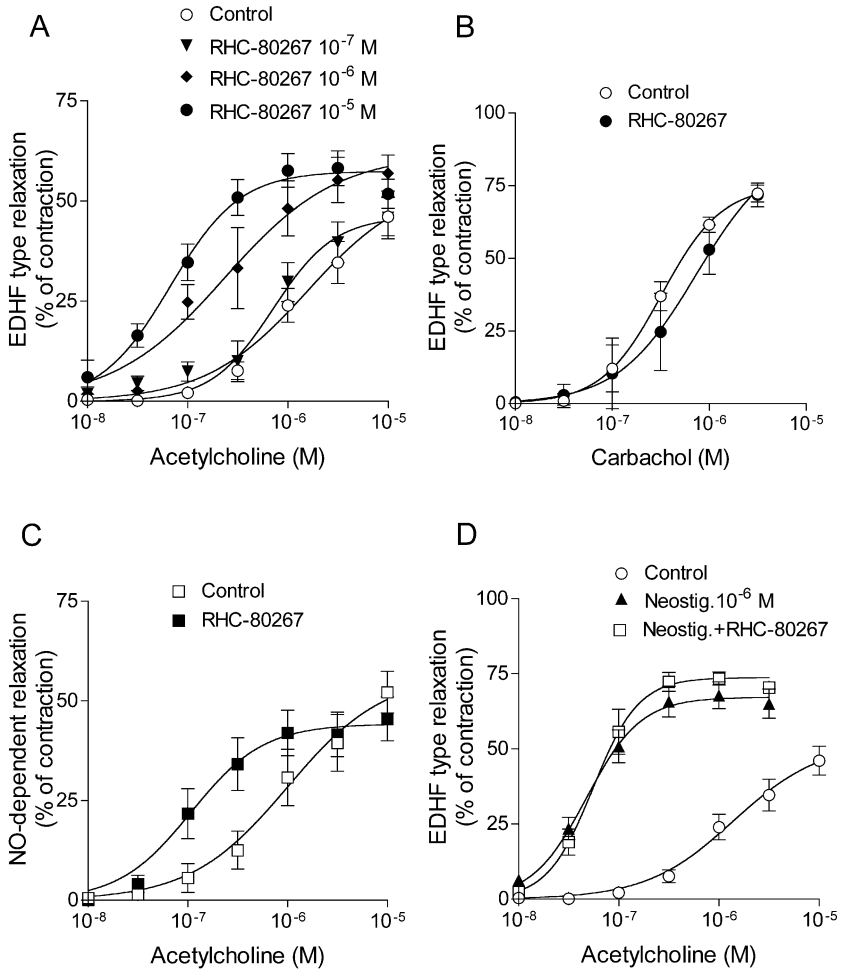

Fig. 1. Effect of RHC-80267 on the endothelium-dependent relaxation of rat mesenteric artery. A. Effect of different concentrations of RHC-80267 on the EDHF type relaxation evoked by acetylcholine in WKY/Kyo@Rj mesenteric arteries contracted with noradrenaline (0.5 μM) in the presence of L-NOArg and indomethacin. B. Effect of RHC-80267 (10 μM) on the EDHF type relaxation evoked by carbachol in WKY/Kyo@Rj mesenteric arteries contracted with noradrenaline (0.5 μM) in the presence of L-NOArg and indomethacin. C. Effect of RHC-80267 (10 μM) on the NO-dependent relaxation evoked by acetylcholine in WKY/Nico mesenteric arteries contracted by 100 mM KCl solution. D. Effect of neostigmine (1 μM) and of neostigmine plus RHC-80267 (10 μM) on the EDHF type relaxation evoked by acetylcholine in WKY/Kyo@Rj mesenteric arteries contracted with noradrenaline (0.5 μM) in the presence of L-NOArg and indomethacin. Mesenteric arteries were preincubated with RHC-80267 and/or neostigmine for 30 min before evoking contraction. Points are means ± S.E.M. from 4–8 determinations.

relaxation curves to acetylcholine, in the absence as in the presence of RHC-80267 (Table 1).

In contrast to the effects of RHC-80267 on the relaxation evoked by acetylcholine, when the EDHF type relaxation was evoked by the nonhydrolysable analogue of acetylcholine, carbachol, preincubation with RHC-80267 (10 μM) did not affect the relaxation (Fig. 1B).

3.2. Effect of RHC-80267 on the NO-dependent relaxation evoked by acetylcholine in mesenteric artery

The NO-dependent relaxation evoked by acetylcholine was tested in arteries contracted with 100 mM KCl solution, in the presence of phentolamine to inhibit the effect of noradenaline that could be released from nerve endings following depolarization. Preincubation with 10 μM RHC-80267 produced a leftward shift of the relaxation evoked by acetylcholine in KCl-contracted arteries: acetylcholine pD_2 values in WKY/Nico rats were 5.98 ± 0.15 ($n = 8$) and 7.00 ± 0.07 ($n = 8$), in the absence and in the presence of 10 μM RHC-80267, respectively ($P < 0.01$) (Fig. 1C).

3.3. Effect of RHC-80267 on the endothelium-independent relaxation in mesenteric artery

The sensitivity to RHC-80267 of endothelium-independent relaxations was tested in the presence of L-NOArg to block endogenous release of NO. RHC-80267 (10 μM) did not change the relaxation evoked by the NO donor SNAP (0.01–10 μM) in arteries contracted with 100 mM KCl (Fig. 2A). The diacylglycerol lipase inhibitor also did not modify the relaxation evoked by the K^+ channel opener cromakalim (0.01–3 μM) in mesenteric arteries contracted with noradrenaline (Fig. 2B).

3.4. Effect of neostigmine on acetylcholine-evoked relaxation

The effect of a cholinesterase inhibitor on the L-NOArg-resistant relaxation evoked by acetylcholine in noradrenaline-contracted

Table 1
Effect of the protein kinase C inhibitors calphostin C and Ro-31-8220, of the phospholipase A_2 inhibitor OBAA, of the analogue of diacylglycerol, 1-oleoyl-2-acetyl-*sn*-glycerol, and of the cholinesterase inhibitor neostigmine on the EDHF type relaxation evoked by acetylcholine in mesenteric artery and its potentiation by RHC-80267

Drug	Noradrenaline-evoked contraction (mN)	Acetylcholine pD_2 ($-\log ED_{50}$ (M))	Noradrenaline-evoked contraction (mN)	Acetylcholine pD_2 ($-\log ED_{50}$ (M))
	–		RHC-80267 (10 μM)	
–	15.8±0.4 (33)	6.41±0.06 (33)	13.5±0.8 (6)	7.22±0.07 (6)[a,b]
Ro-31-8220 (1 μM)	14.8±0.7 (4)	6.17±0.12 (4)	11.5±1.0 (4)	7.25±0.25 (4)[a,b]
Calphostin C (1 μM)	10.3±3.1 (4)[c]	6.22±0.04 (4)	8.1±1.6 (4)[c]	7.38±0.21 (4)[a,b]
OBAA (5 μM)	14.9±1.0 (4)	6.17±0.11 (4)	12.4±1.1 (4)	7.28±0.17 (6)[a,b]
1-Oleoyl-2-acetyl-*sn*-glycerol (80 μM)	21.1±2.8 (3)	6.22±0.03 (3)	n.d.	n.d.
Neostigmine (1 μM)	15.3±1.1 (4)	7.34±0.04 (4)[a]	15.9±0.8 (3)	7.29±0.09 (3)[a]

pD_2 values are means of the individual pD_2 [$-\log ED_{50}$ (M)] values measured by non-linear regression of the concentration–effect curves to acetylcholine in noradrenaline-contracted mesenteric artery from WKY/Nico rats. L-NOArg (100 μM) and indomethacin (10 μM) were present in all solutions. Pre-constriction level evoked by noradrenaline (0.5 μM) is expressed in millinewton. The number of curves is indicated between parenthesis. [a]$P<0.05$ versus the pD_2 value obtained in the absence of drug, [b]$P<0.05$ versus the pD_2 value obtained in the absence of RHC-80267, [c]$P<0.05$ versus the contraction measured in the absence of drug. n.d., not determined.

arteries was tested by performing the concentration–relaxation curves in the presence of neostigmine, an inhibitor of cholinesterase (Iwanaga et al., 1990). After incubation with neostigmine (1 μM), the concentration–relaxation curve to acetylcholine was significantly shifted to the left. After incubation in the presence of neostigmine (1 μM) and RHC-80267 (10 μM), the concentration–relaxation curve to acetylcholine was not different from the curve obtained in the presence of neostigmine alone (Fig. 1D, Table 1). Neostigmine similarly potentiated the NO-dependent relaxation evoked by acetylcholine in KCl-contracted arteries but did not change the relaxation evoked by carbachol (data not shown).

3.5. Effect of RHC-80267 on the cholinesterase activity

The in vitro effect of RHC-80267 on cholinesterase was tested in rat brain homogenate and compared to the inhibition produced by neostigmine. RHC-80267 inhibited cholinesterase activity concentration-dependently with a pIC_{50} value of 5.39 ±0.06 (IC_{50} 4 μM) (Fig. 3). The concentration–effect curve of RHC-80267 was parallel to the curve obtained with neostigmine but RHC-80267 was about 1000-fold less potent than neostigmine, which had a pIC_{50} value of 8.54±0.06 (IC_{50} 2.9 nM), a value close to reported data (Iwanaga et al., 1990).

4. Discussion

The present results show that RHC-80267, which is commonly used as an inhibitor of diacylglycerol lipase (Mason-Garcia et al., 1992; Nishimaru et al., 2003; Shinoda et al., 1997; Suzuki et al., 2002; Venkatachalam et al., 2003), displays significant anti-cholinesterase activity, which was responsible for the potentiation of the endothelium-dependent relaxation to acetylcholine in rat mesenteric artery.

RHC-80267 displayed a selective effect on the endothelium-dependent relaxation evoked by acetylcholine. Neither the relaxation evoked by the NO donor SNAP nor the relaxation evoked by cromakalim, which directly hyperpolarizes smooth muscle cells by opening ATP-dependent K^+ channels, was affected. Acetylcholine relaxes rat mesenteric artery through two main endothelium-dependent pathways: an NO-mediated relaxation, which is blocked by nitric oxide synthase (NOS) inhibitors like L-NOArg, and an EDHF-mediated relaxation, which is observed in the presence of NOS inhibitors and is abolished

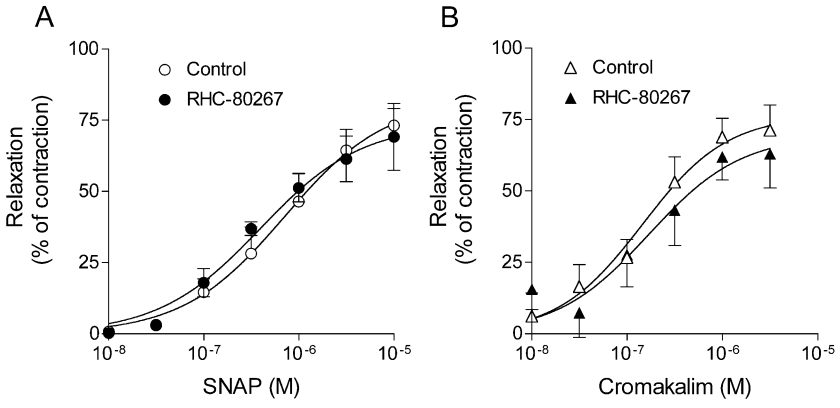

Fig. 2. Effect of RHC-80267 on the endothelium-independent relaxation in rat mesenteric artery. A. Relaxation was evoked by the NO donor SNAP in arteries contracted by 100 mM KCl solution in the presence of L-NOArg. B. Relaxation was evoked by the K^+ channel opener cromakalim in arteries contracted by noradrenaline (0.5 μM) in the presence of L-NOArg. Mesenteric arteries from WKY/Nico rats were preincubated with RHC-80267 (10 μM) for 30 min before evoking contraction. Points are means±S.E.M. from 4–6 determinations.

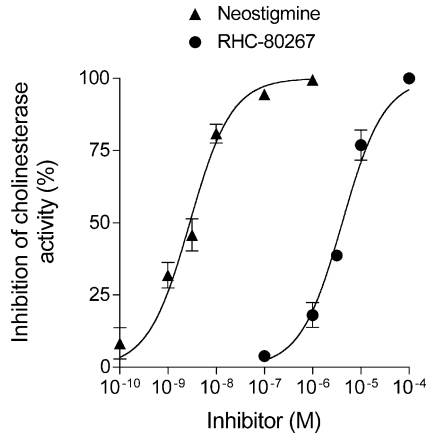

Fig. 3. In vitro inhibition of cholinesterase by RHC-80267 and neostigmine. Cholinesterase activity was measured in rat brain homogenate with acetylthiocholine (0.3 mM). Points are means±S.E.M. from 5–6 determinations.

by high KCl solution or by the K^+ channel blockers charybdotoxin and apamin (Ghisdal et al., 1999; Ghisdal and Morel, 2001). In the present study, both the NO-dependent relaxation and the EDHF type relaxation evoked by acetylcholine were significantly potentiated in the presence of the diacylglycerol lipase inhibitor. Inhibition of diacylglycerol lipase has two potential effects: an accumulation of diacylglycerol and a decrease in arachidonic acid, the degradation product of diacylglycerol. Diacylglycerol is released together with IP_3 after hydrolysis of PIP_2 by phospholipase C. It is known to activate protein kinase C but has also been reported to regulate cation channels in a protein kinase C-independent manner (Venkatachalam et al., 2003). Contribution of protein kinase C to the potentiation of acetylcholine relaxation by RHC-80267 was excluded since the effect of RHC-80267 was not affected by the protein kinase C inhibitors calphostin C and Ro-31-8220. Moreover, the diacylglycerol analogue, 1-oleoyl-2-acetyl-*sn*-glycerol, had no effect on the relaxation to acetylcholine. Change in the relaxation to acetylcholine through an interaction with arachidonic acid level was another interesting hypothesis since arachidonic acid has been proposed to be involved in the EDHF-pathway (Fleming, 2004). However, a decrease in its concentration consecutive to the inhibition of diacylglycerol lipase should depress the EDHF-dependent relaxation. In addition, inhibition of phospholipase A_2 with OBAA, which should also reduce the release of arachidonic acid, did not affect the relaxation evoked by acetylcholine. Moreover, the two pathways of relaxation activated by acetylcholine in mesenteric artery, namely, the NO-dependent and the EDHF type relaxations, were similarly potentiated by RHC-80267, suggesting that diacylglycerol lipase inhibition should interfere with a step that is common to the two pathways.

Two observations indicated that the potentiation by RHC-80267 of the relaxation to acetylcholine could be caused by the inhibition of cholinesterase: (1) RHC-80267 did not affect the responses to carbachol, the carbamyl derivative of acetylcholine which only differs from acetylcholine by its resistance to hydrolysis by cholinesterase, and (2) the effect of RHC-80267 was mimicked by the inhibitor of cholinesterase neostigmine, RHC-80267 showing no additional effect in the presence of a maximally effective concentration of neostigmine. The determination of the effect of RHC-80267 on the cholinesterase activity of brain homogenate confirmed that RHC-80267 inhibited this enzyme in the same range of concentrations increasing the relaxation to acetylcholine, namely at 1–10 μM. Elements of the neuronal and non-neuronal cholinergic systems have been shown to be present in the vascular wall (Amenta et al., 1981; Cohen et al., 1979; Kirkpatrick et al., 2001) and regulation of acetylcholine activity by cholinesterase has been described in several arteries (Altiere et al., 1994; Choy et al., 2002). By preventing the continuous degradation of acetylcholine, cholinesterase inhibitors increase the availability of the agonist at the level of the endothelial muscarinic receptor.

In conclusion, the potentiating effect of RHC-80267 on the endothelium-dependent relaxation evoked by acetylcholine in rat mesenteric artery is due to its anti-cholinesterase activity. Since IC_{50} values of 4–5 μM have been reported for the inhibition of diacylglycerol lipase by RHC-80267 in platelets (Amin et al., 1986; Moriyama et al., 1999; Sutherland and Amin, 1982), the present data indicate that RHC-80267 has a similar potency on cholinesterase and on diacylglycerol lipase. Critical evaluation of the data obtained with this drug is thus needed before its effect can be attributed to diacylglycerol lipase inhibition.

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References

Altiere, R.J., Travis, D.C., Thompson, D.C., 1994. Characterization of acetylcholinesterase in rabbit intrapulmonary arteries. Am. J. Physiol. 267, L745–L752.

Amenta, F., Cavallotti, C., Ceccarelli, E., Evangelisti, F., 1981. Cholinergic nerves in the mesentery. Acta Histochem. 69, 125–131.

Amin, D., Sutherland, C.A., Khandwala, A.S., Jamall, I.S., Kapoor, A.L., 1986. Inhibition of the effects of thrombin on guinea pig platelets by the diacylglycerol lipase inhibitor RHC 80267. Thromb. Res. 44, 75–84.

Bores, G.M., Huger, F.P., Petko, W., Mutlib, A.E., Camacho, F., Rush, D.K., Selk, D.E., Wolf, V., Kosley Jr., R.W., Davis, L., Vargas, H.M., 1996. Pharmacological evaluation of novel Alzheimer's disease therapeutics: acetylcholinesterase inhibitors related to galanthamine. J. Pharmacol. Exp. Ther. 277, 728–738.

Choy, W.Y., Wong, Y.F., Kwan, Y.W., Au, A.L., Lau, W.H., Raymond, K., Zuo, J.Z., 2002. Role of mitogen-activated protein kinase pathway in acetylcholine-mediated in vitro relaxation of rat pulmonary artery. Eur. J. Pharmacol. 434, 55–64.

Cohen, M.L., Landry, A.S., Hemrick, S.K., Fuller, R.W., 1979. Norepinephrine, monoamine oxidase, and acetylcholinesterase in the rat jugular vein compared with other blood vessels. Can. J. Physiol. Pharm. 57, 1246–1250.

Feletou, M., Vanhoutte, P.M., 1988. Endothelium-dependent hyperpolarization of canine coronary smooth muscle. Br. J. Pharmacol. 93, 515–524.

Fleming, I., 2004. Cytochrome P450 epoxygenases as EDHF synthase(s). Pharmacol. Res. 49, 525–533.

Ghisdal, P., Morel, N., 2001. Cellular target of voltage and calcium-dependent K(+) channel blockers involved in EDHF-mediated responses in rat superior mesenteric artery. Br. J. Pharmacol. 134, 1021–1028.

Ghisdal, P., Godfraind, T., Morel, N., 1999. Effect of nitro-L-arginine on electrical and mechanical responses to acetylcholine in the superior mesenteric artery from stroke-prone hypertensive rat. Br. J. Pharmacol. 128, 1513–1523.

Iwanaga, Y., Miyashita, N., Morikawa, K., Mizumoto, A., Kondo, Y., Itoh, Z., 1990. A novel water-soluble dopamine-2 antagonist with anticholinesterase activity in gastrointestinal motor activity. Comparison with domperidone and neostigmine. Gastroenterology 99, 401–408.

Kirkpatrick, C.J., Bittinger, F., Unger, R.E., Kriegsmann, J., Kilbinger, H., Wessler, I., 2001. The non-neuronal cholinergic system in the endothelium: evidence and possible pathobiological significance. Jpn. J. Pharmacol. 85, 24–28.

Konrad, R.J., Major, C.D., Wolf, B.A., 1994. Diacylglycerol hydrolysis to arachidonic acid is necessary for insulin secretion from isolated pancreatic islets: sequential actions of diacylglycerol and monoacylglycerol lipases. Biochemistry 33, 13284–13294.

Mason-Garcia, M., Clejan, S., Tou, J.S., Beckman, B.S., 1992. Signal transduction by the erythropoietin receptor: evidence for the activation of phospholipases A2 and C. Am. J. Physiol 262, C1197–C1203.

Moncada, S., Vane, J.R., 1978. Pharmacology and endogenous roles of prostaglandin endoperoxides, thromboxane A2, and prostacyclin. Pharmacol. Rev. 30, 293–331.

Moriyama, T., Urade, R., Kito, M., 1999. Purification and characterization of diacylglycerol lipase from human platelets. J. Biochem. 125, 1077–1085 (Tokyo).

Nishimaru, K., Tanaka, Y., Tanaka, H., Shigenobu, K., 2003. Pharmacological evidence for involvement of phospholipase D, protein kinase C, and sodium–calcium exchanger in alpha-adrenoceptor-mediated negative inotropy in adult mouse ventricle. J. Pharm. Sci. 92, 196–202.

Palmer, R.M., Ferrige, A.G., Moncada, S., 1987. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. Nature 327, 524–526.

Shinoda, J., Suzuki, A., Oiso, Y., Kozawa, O., 1997. Involvement of phosphatidylcholine hydrolysis by phospholipase D in extracellular ATP-induced arachidonic acid release in aortic smooth muscle cells. Arterioscler. Thromb. Vasc. Biol. 17, 295–299.

Shlykov, S.G., Sanborn, B.M., 2004. Stimulation of intracellular Ca^{2+} oscillations by diacylglycerol in human myometrial cells. Cell Calcium 36, 157–164.

Sutherland, C.A., Amin, D., 1982. Relative activities of rat and dog platelet phospholipase A2 and diglyceride lipase. Selective inhibition of diglyceride lipase by RHC 80267. J. Biol. Chem. 257, 14006–14010.

Suzuki, H., Kito, Y., Fukuta, H., Yamamoto, Y., 2002. Effects of RHC-80267, an inhibitor of diacylglycerol lipase, on excitation of circular smooth muscle of the guinea-pig gastric antrum. J. Smooth Muscle Res. 38, 153–164.

Taylor, S.G., Weston, A.H., 1988. Endothelium-derived hyperpolarizing factor: a new endogenous inhibitor from the vascular endothelium. Trends Pharmacol. Sci. 9, 272–274.

Venkatachalam, K., Zheng, F., Gill, D.L., 2003. Regulation of canonical transient receptor potential (TRPC) channel function by diacylglycerol and protein kinase C. J. Biol. Chem. 278, 29031–29040.