J. Pharm. Pharmacol. 1989, 41: 347–349
Received June 29, 1988

RS-61756-007: a potent and selective thromboxane receptor (TP) agonist

R. M. EGLEN, R. L. WHITING, *Institute of Pharmacology, Syntex Research, 3401 Hillview Ave., Palo Alto, CA 94304, USA*

Abstract—The activity of RS-61756-007 (methyl-9-oxo, 15 α-hydroxy, 16-phenoxy, 17, 18, 19, 20-tetranor prosta 4, 5, 13(E) trienoate, 4, 5, 6 (R), 8(R)) has been assessed at prostanoid receptors (DP, EP_1, EP_2, FP, IP and TP) in-vitro. The activity profile of RS-61756-007 resembles that of U46619, in that agonism was observed at TP and to a lesser extent at FP receptors, but there was no activity at the remaining subtypes. The actions of both RS-61756-007 and U46619 were antagonized in a similar manner by the TP antagonist SQ 29,548. We conclude that RS-61756-007 is a highly potent TP agonist.

Prostanoid receptors have been classified into at least 5 subtypes (Kennedy et al 1982). The prostanoid receptor stimulated by thromboxane A_2 (TxA_2) has been designated as TP (for review see Coleman et al 1985), and has been extensively characterized both in-vivo and in-vitro. A selective TP agonist, U46619 has been shown to mimic the actions of TxA_2 (Coleman et al 1981), and a number of selective, competitive TP antagonists have also been described including BM 13,177 (Stegmeier et al 1984), AH 23848 (Brittain et al 1985), and SQ 29,548 (Ogletree et al 1985). In addition, a number of in-vitro preparations have been studied which are highly sensitive to the effects of TP receptor stimulation, including the rat and rabbit aorta (Coleman et al 1981, Jones et al 1982), the guinea-pig trachea (Jones et al 1982) and the dog saphenous vein (Jones et al 1982). These and other studies have led to a characterization of the TP receptor and consequently the role of TP receptor stimulation in a number of cardiovascular disorders (Brittain et al 1985).

In the present study we have characterized the receptor profile of RS-61756-007 (methyl-9-oxo,15α-hydroxy,16-phenoxy,17,18,19,20-tetranor prosta 4,5,13(E)trienoate, 4,5,6(R),8(R)). This compound (Fig. 1) acts as a highly potent and selective TP agonist, and should prove a useful agent in the classification of prostanoid receptors.

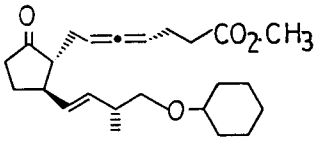

FIG. 1. RS 61756-007.

Materials and methods

Tissue preparation. Smooth muscle tissues were isolated from either male Dunkin-Hartley guinea-pigs (300–350 g) or male Sprague-Dawley rats (250–300 g), previously killed by CO_2 inhalation. Platelets were prepared from blood removed from male Dunkin-Hartley guinea-pigs (450–550 g), previously anaesthetized using sodium pentobarbitone (Sagatal: 60 mg kg^{-1} i.p.).

Smooth muscle preparations. The methods used were similar to those previously described by Coleman et al (1981). Guinea-pig and rat thoracic aortae were isolated and cut in a helical fashion (Furchgott & Bhadrakom 1953). The spiral strips were placed under 1·0 g tension in Krebs physiological salt solution (pH 7·4, 37°C, gassed with 5% CO_2 in O_2). Sixty min was allowed for equilibration during which time the bathing fluid was replaced every 15 min.

Guinea-pig proximal ileum (Horton & Main 1973) were removed and gently flushed with warm Tyrode physiological salt solution. The tissues were then suspended, under 1·0 g tension in Tyrode solution (pH 7·4, 37°C, gassed with 5% CO_2 in O_2) and allowed 60 min to equilibrate, during which time the bathing fluid was replaced every 15 min.

Guinea-pig tracheae were isolated, cleaned of all adhering connective tissue and cut in a zig-zag fashion, using the method of Emmerson & MacKay (1979). The tissues were then suspended under 1·0 g tension in Krebs physiological salt solution, in a similar manner to that described for the aortic preparations. In experiments in which tracheal relaxation was studied, the resting tension was increased by the addition of carbachol (1×10^{-5} M).

Ascending colon muscle (1·5 cm) was prepared from rats according to Eglen & Whiting (1988) and placed in Tyrode physiological salt solution under 1·0 g tension. The Tyrode solution contained $2{\cdot}8 \times 10^{-6}$ M SQ 29,548 to inhibit TP receptor activity (Eglen & Whiting 1988).

Platelets. Platelets were prepared according to Hamid-Bloomfield & Whittle (1986). Samples of whole guinea-pig blood were added to trisodium citrate (0·38 g% w/v final concentration) and centrifuged at 200 *g* for 10 min. The supernatant was aspirated, and respun again at 200 *g* for 10 min. The supernatant was aspirated to form the platelet rich plasma (PRP). The remaining fractions were centrifuged at 11 000 *g* for 2 min, and the resulting supernatant formed the platelet poor plasma (PPP). Samples (250 μL) of PRP and PPP were placed in siliconized glass cuvettes, maintained at 37°C and continuously stirred at 2000 rev min^{-1}.

Experimental protocols. In all preparations, responses were characterized initially to standard agonists at each receptor subtype i.e. DP-BW 245C (platelet); EP_1-PGE_1 (ileum); EP_2-PGE_2 (trachea); FP-$PGF_{2\alpha}$ (colon); IP-PGI_2 (platelet) and TP-U46619 (aorta, trachea and platelet). PGD_2, PGE_2, $PGF_{2\alpha}$, PGI_2 and TxA_2 are the most potent endogenous prostaglandins for the DP, EP, FP, IP and TP subtypes, respectively. EP receptors have been shown to exist in at least two subtypes denoted as EP_1 and EP_2. Only responses to the former are antagonized by either AH 6809 or SC-19920 (Coleman et al 1985).

Smooth muscle. Cumulative concentration-response curves were constructed to either U46619 or RS-61756-007, using the aortic and tracheal preparations. Agonist concentrations were added at concentrations 0·5 log units apart, and increasing concentrations were added once a sustained equilibrium response was attained to the previous concentration (3–8 min). In experiments using the ileum, agonists were added on a non-cumulative basis for 30 s, on a 5 min dose cycle.

In experiments in which the TP antagonist, SQ 29,548 (Ogletree et al 1985) was used, the tissues were equilibrated for 60 min, before construction of a concentration-response curve.

Correspondence to R. M. Eglen, Institute of Pharmacology, Syntex Research, 3401 Hillview Ave., Palo Alto, CA 94304, USA.

Each tissue was exposed to only one antagonist concentration, and a control tissue in which no antagonist was used was always run to correct for any sensitivity changes.

Platelets. The extent of aggregation of each sample of PRP was assessed, by the addition of ADP ($2{\cdot}8 \times 10^{-6}$ M). Concentration-response curves were then constructed to the agonist, using a new sample for each concentration. All samples were allowed 2 min for equilibration, before the addition of the aggregating agent.

Analysis of Results

Smooth muscle. All contractions were recorded isometrically (mg) using Hugo Sach KSE 30 force transducer connected to a 4 channel Graphtec-Watanabe recorder. The contractions at each agonist concentration were normalized and the potency (EC50) determined. The dissociation constants for the TP antagonist, SQ 29,548, were determined using the method of Arunlakshana & Schild (1959). A Schild slope which was not significantly different from unity was considered to be consistent with competitive antagonism.

Platelets. Platelet aggregation was measured using a Chrono-log Whole-Blood Aggregometer and changes in light transmission recorded using a flatbed recorder (Born 1962). The extent of aggregation was determined using the primary aggregation wave, occurring within 3 min. Responses, determined in duplicate, were normalized, and the EC50 value calculated.

Krebs solution. (mmol L^{-1}): NaCl, 118·4, KCl, 4·9, $MgSO_4{\cdot}7H_2O$, 1·2, KH_2PO_4, 1·2, glucose, 11·1, $NaHCO_3$, 25·0, $CaCl_2{\cdot}6H_2O$ 2·5.

Tyrode solution. (mmol L^{-1}): NaCl, 136·9, KCl 2·7, $MgCl_2{\cdot}6H_2O$, 1·1, $NaH_2PO_4{\cdot}2H_2O$, 0·4, glucose, 5·6 $NaHCO_3$, 11·9, $CaCl_2{\cdot}6H_2O$, 1·8.

All solutions contained phenoxybenzamine (7×10^{-7} mol L^{-1}) and indomethacin ($2{\cdot}8 \times 10^{-6}$ mol L^{-1}). Atropine (1×10^{-6} mol L^{-1}) was also present in all solutions, except in those experiments in which the tone of the isolated trachea was increased by the addition of carbachol (see above).

Drugs used. Atropine, (Sigma), BW 245C (5-(6-carboxyhexyl)-1(3-cyclohexyl-3-hydroxypropyl) hydantoin, Burroughs Wellcome), carbachol (Sigma), indomethacin (Sigma), phenoxybenzamine (SK & F), PGD_2, PGE_1, PGE_2, $PGF_{2\alpha}$, PGI_2, U46619 (9, 11-methanoepoxy PGH_2, Cayman Chemical Co. Ltd), and SQ 29,548 (15-[1α, 2β(52),3β, 4α]-7-[z-(phenylamino)-carbonyl] hydrazino]methyl-7-oxobicyclo[2·21]-hept-2yl]-5-heptenoic acid, Squibb). RS-61756-007 was synthesized in the Institute of Organic Chemistry, Syntex, Palo Alto.

Results

Agonist Studies. U46619 or RS-61756-007, from 1×10^{-12} to 1×10^{-6} M, elicited concentration-dependent contractions of the guinea-pig aorta or trachea or the rat aorta. The potencies (−log EC50) are shown in Table 1. In all of these preparations, RS-61756-007 was significantly ($P < 0{\cdot}05$) more potent than U46619.

However, no contractile response was observed to either U46619 or RS-61756-007 at EP receptors in the guinea-pig isolated ileum at the concentrations examined ($1 \times 10^{-11} - 1 \times 10^{-5}$ M). In contrast, concentration-dependent contractions were observed to PGE_1 in this preparation (−log EC50 = 7·81 ± 0·05, mean ± s.e.m., n = 4). U46619 or RS-61756-007, in the presence of $2{\cdot}8 \times 10^{-6}$ M SQ 29,548 (to exclude TP agonist activity), did not elicite relaxations at EP_2 receptors present in the guinea-pig trachea (previously precontracted with carbachol 1×10^{-5} M). However, concentration-dependent relaxations were observed to PGE_2 (−log EC50 = 6·24 ± 0·12, mean ± s.e.m., n = 6). RS-61756-007 and U46619 elicited concentrations of the rat colon, in the presence of $2{\cdot}8 \times 10^{-6}$ M SQ 29,548 (to exclude TP agonist activity). The −log EC50 values for RS-61756-007 and U46619 were 6·55 ± 0·13 and 5·93 ± 0·14 (mean ± s.e.m., n = 6), respectively.

Table 1. Potencies (−log EC50) of U46619 and RS-61756-007 at TP receptors in-vitro.

Preparation	U46619	RS-61756-007
Guinea-pig trachea	7·64 ± 0·12 (6)	8·82 ± 0·10 (7)
Guinea-pig aorta	7·32 ± 0·08 (4)	9·28 ± 0·15 (3)
Guinea-pig PRP[a]	7·53 ± 0·09 (6)	8·91 ± 0·15 (4)
Rat-aorta	7·53 ± 0·13 (5)	8·62 ± 0·13 (9)

Values are mean ± s.e.m., number of animals in parentheses.
[a] PRP-platelet rich plasma.

In the guinea-pig platelet rich plasma, U46619 and RS-61756-007 acted as potent inducers of aggregation. In agreement with findings in the isolated aortic and tracheal preparations, RS-61756-007 was significantly more potent than U46619. These data are shown in Table 1.

In the presence of 4×10^{-6} M SQ 29,548, to exclude TP agonist activity, both U46619 and RS-61756-007 did not inhibit aggregation induced by ADP at the concentrations examined ($1 \times 10^{-12} - 3 \times 10^{-5}$ M). However, concentration-dependent inhibition of ADP-induced aggregation was observed with PGI_2 (−log EC50 = 9·1 ± 0·08) and BW245C (−log EC50 = 8·0 ± 0·12). Values are mean ± s.e.m., n = 4–8.

Antagonist studies. Contractile responses to both U46619 and RS-61756-007 were antagonized competitively by SQ 29,548 since parallel dextral shifts in the concentration-response curves were observed, with no decrease in the maximum. The slopes of the Schild plots were not significantly ($P < 0{\cdot}05$) different from unity, except when RS-61756-007 was used as the agonist at TP receptors in the rat aorta. These data are shown in Table 2. The pA_2 values obtained with U46619 and RS-61756-007 were similar in all three preparations, again with the exception of the pA_2 obtained at TP receptors in the rat aorta, using RS-61756-007 as the agonist (Table 2).

Table 2. pA_2 values and Schild plots for U46619 and RS-61756-007 at TP receptors in-vitro.

Preparation	pA_2	Schild slope
a. U46619		
Guinea-pig aorta	8·40 ± 0·09	0·82 ± 0·12
Rat-aorta	8·38 ± 0·05	0·95 ± 0·08
Guinea-pig trachea	8·49 ± 0·05	0·89 ± 0·12
b. RS-61756-007		
Guinea-pig aorta	8·38 ± 0·07	0·91 ± 0·02
Rat-aorta	8·81 ± 0·09	0·79 ± 0·02
Guinea-pig trachea	8·38 ± 0·05	1·10 ± 0·02

Values are mean ± s.e.m. n = 6–10.

Discussion

U46619, a selective TP agonist, has proved to be a useful tool in the classification of prostanoid receptors (Coleman et al 1981; Kennedy et al 1982). In addition, the development of potent and selective TP receptor agonists, such as SQ 29,548, (Ogletree et al

1985) have also allowed rigorous classification of the TP receptor. In the present study, we have characterized the actions of RS-61756-007, a compound which also appears to act as a selective TP agonist.

U46619 and RS-61756-007 exhibited similar receptor profiles, since they did not stimulate EP_1 receptors which mediated contraction of the ileum or EP_2 receptors which mediated relaxations of the trachea (see Coleman et al 1985 for review). These data using U46619 are in agreement with previous studies (Coleman et al 1981). In addition, neither U46619 or RS-61756-007 stimulated DP or IP receptors in platelets, since no inhibition of ADP-induced aggregation was observed. In all of these assays, appropriate responses were observed to PGE_1, PGE_2, BW 245C and PGI_2 respectively, however. U46619 and RS-61756-007, therefore, lack agonism at DP, EP_1, EP_2 and IP prostanoid receptors. Similar data using U46619 have been previously reported (Coleman et al 1981). It should be noted that Coleman et al (1987) have described a novel EP subtype denoted as EP_3. No data are currently available about the effect of RS-61756-007 at this receptor.

However, U46619 and RS-61756-007, elicited concentration-dependent contractions of the guinea-pig aorta and trachea, in addition to the rat aorta. These preparations have been previously shown to contract in response to TP receptor stimulation (Jones et al 1982). In agreement with those results, both U46619 and RS-61756-007, potently induced platelet aggregation, a response also been shown to result from TP receptor stimulation (Ogletree et al 1985).

The use of the specific TP antagonist SQ 29,548 (Ogletree et al 1985) confirms the proposal that both U46619 and RS-61756-007 act at TP receptors. Similar equilibrium dissociation constants, as estimated by the pA_2 value, were obtained using both U46619 and RS-61756-007 in the aortic and tracheal preparations. The pA_2 values were similar to those previously reported (Ogletree et al 1985) using U46619 as the agonist.

It is apparent, therefore, that both U46619 and RS-61756-007 are potent TP agonists. However, RS-61756-007 was consistently more potent than U46619. This is unlikely to result from TP heterogeneity, since the pA_2 values for SQ 29,548 were similar, and therefore may result from either a greater intrinsic efficacy or a higher TP receptor affinity of RS-61756-007 in comparison with U46619.

Although RS-61756-007 acted as a highly potent TP receptor agonist it was as selective as U46619, since no agonism to either agonist was observed at EP_1, EP_2, DP or IP receptors. Although the compound may have acted as a weak FP agonist at receptors in the rat colon, an alternative explanation is that RS-61756-007 at high concentrations overcame the TP antagonism in these studies. Further experiments need to be done using other FP assays such as the dog or cat iris muscle (Dong & Jones 1982).

In conclusion, RS-61756-007 acts as a highly potent TP receptor agonist, and it should prove a useful tool in the classification of TP receptors in particular and prostanoid receptors in general.

References

Arunlakshana, O., Schild, H. O. (1959). Some quantitative uses of drug antagonists. Br. J. Pharmacol. Chemother. 14: 48–58

Born, G. V. R. (1962) Quantitative investigations into the aggregation of blood platelets. J. Physiol. 162, 67P

Brittain, R. T., Boutal, L., Carter, M. C., Coleman, R. A., Collington, E. W., Geisow, H. P., Hallett, P., Hornby, E. J., Humphrey, P. P. A., Jack, D., Kennedy, I., Lumley, P., McCabe, P. J., Skidmore, I. F., Thomas, M., Wallis, C. J. (1985) AH 23848: A thromboxane-receptor blocking drug that can clarify the pathophysiologic role of thromboxane A_2. Circulation 72: 1208–1218

Coleman, R. A., Humphrey, P. P. A., Kennedy, I., Levy, G. P., Lumley, P. (1981) Comparison of the actions of U46619, a prostaglandin H_2 analogue, with those of prostaglandin H_2 and thromboxane A_2 on some isolated smooth muscle preparations. Br. J. Pharmacol. 73: 773–778

Coleman, R. A., Humphrey, P. P. A., Kennedy, I. (1985) Prostanoid receptors in smooth muscle: Further evidence for a proposed classification. Trends in Autonomic Pharmacol. Volume 3, Ed. S. Kalsner, Taylor & Francis, London, 35-49

Coleman, R. A., Kennedy, I., Sheldrick, R. L. G., Tolowinska, I. Y. (1987) Further evidence for the existence of three subtypes of PGE_2-sensitive (EP$-$) receptors. Br. J. Pharmacol. 91: 323P

Dong, Y. J., Jones, R. L. (1982) Effect of prostaglandins and thromboxane analogues on bullock and dog iris sphincter preparations. Ibid. 76: 149–155

Emmerson, J., MacKay, D. (1979). The zig-zag tracheal strip. J. Pharm. Pharmacol. 31: 798

Eglen, R. M., Whiting, R. L. (1988). The action of prostanoid receptor agonists and antagonists on smooth muscle and platelets. Br. J. Pharmacol. 94: 591–601

Furchgott, R. F., Bhadrakom, S. (1953) Reactions of the rabbit aorta to epinephrine, isopropylarterenol, sodium nitrite and other drugs. J. Pharmacol. Exp. Ther. 108: 129–143

Hamid-Bloomfield, S., Whittle, B. J. R. (1986) Prostaglandin D_2 interacts at thromboxane receptor sites on guinea pig platelets. Br. J. Pharmacol. 88: 931–936

Hamid, S., Whittle, B. J. R. (1985) Interaction of prostaglandin D_2 with prostacyclin carbacyclin and hydantoin prostaglandin, BW 245C in guinea pig platelets. Ibid. 85: 285–290

Horton, E. W., Main, I. H. M. (1973) A comparison of the actions of prostaglandins $F_{2\alpha}$ and E_1 on smooth muscle. Br. J. Pharmacol. Chemother. 24: 470–476

Jones, R. L., Peesapate, V., Wilson, N. M. (1982) Antagonism of the thromboxane-sensitive contractile systems of the rabbit aorta, dog saphenous vein and guinea pig trachea. Br. J. Pharmacol. 76, 423–438

Kennedy, I., Coleman, R. A., Humphrey, P. P. A., Levy, G. P., Lumley, P. (1982) Studies on the characterisation of prostanoid receptors: a proposed classification. Prostaglandins 24: 667–689

Ogletree, M. L., Harris, D. N., Greenberg, R., Haslanger, M. F., Nakane, M. (1985) Pharmacological actions of SQ 29,548, a novel selective thromboxane antagonist. J. Pharm. Exp. Ther. 234: 435–441

Stegmeier, K., Pill, J., Müller-Beckman, B., Schmidt, F. H., Witte, E-C., Wolff, H-P., Patscheke, H. (1984) The pharmacological profile of the thromboxane A_2 antagonist, BM 13,177. A new anti-platelet and anti-thrombotic drug. Thromb. Res., 35: 379–395