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European Journal of Pharmacology 334 (1997) 241–247

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Ketotifen prevents gastric hyperemia induced by intracisternal thyrotropin-releasing hormone at a low dose

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Received 9 June 1997; revised 15 June 1997; accepted 18 July 1997

Abstract

The thyrotropin-releasing hormone (TRH) analog, RX 77368, (*p*-Glu–His-(3,3′-dimethyl)-Pro-NH_2) injected intracisternally (i.c.) at low doses increases gastric mucosal blood flow through vagal cholinergic and calcitonin gene-related peptide dependent pathways. The influence of the mast cell stabilizer, ketotifen, on i.c. injection of RX 77368 (1.5 ng)-induced changes in gastric mucosal blood flow (hydrogen gas-clearance technique), gastric acid secretion and mean arterial pressure was studied in urethane-anesthetized rats. RX 77368 increased gastric blood flow by 131% and systemic arterial pressure by 11 mm Hg and decreased gastric mucosal vascular resistance by 54% whereas acid secretion was not altered within the 30 min period post injection. Ketotifen had no effect on these basal parameters but abolished i.c. RX 77368-induced increased gastric mucosal blood flow and decreased gastric vascular resistance. These data suggest that mast cells may be part of the peripheral mechanisms involved in vagal gastric hyperemia induced by TRH analog injected i.c. at a low dose. © 1997 Elsevier Science B.V.

Keywords: Acid secretion; Gastric mucosal blood flow; Ketotifen; Mast cell; Mean arterial pressure; RX 77368; TRH (thyrotropin-releasing hormone); Vagus; Brain–gut

1. Introduction

Thyrotropin releasing hormone (TRH) in the brainstem plays a physiological role in the vagal stimulation of gastric function and resistance of the gastric mucosa to injury (Taché and Yang, 1994; Kaneko et al., 1995a; Kaneko et al., 1995b). In particular, intracisternal (i.c.) or dorsal motor nucleus injection of TRH or the stable TRH analog, RX 77368, (*p*-Glu–His-(3,3′-dimethyl)-Pro-NH_2), at doses subthreshold to stimulate gastric acid secretion, increases gastric mucosal blood flow and protects the gastric mucosa against ethanol-induced gastric injury in rats (Yoneda and Taché, 1992; Király et al., 1993; Cardin et al., 1995; Kato et al., 1995; Király et al., 1997b). We recently demonstrated that the gastric mucosal hyperemia and gastric protection induced by i.c. RX 77368 at 1.5 ng are mediated by vagal muscarinic dependent pathways and calcitonin gene-related peptide (CGRP) contained in capsaicin-sensitive afferent fibers (Yoneda and Taché, 1992; Kato et al., 1994; Kato et al., 1995; Király et al., 1997b). The mechanisms recruiting the 'local effector function' of capsaicin-sensitive afferent nerve endings (Holzer, 1988; Maggi and Meli, 1988) in the stomach after central activation of gastric vagal efferent discharges by RX 77368 injected i.c. at a low dose (O-Lee et al., 1997) are still unknown.

Earlier studies indicate that electrical vagal stimulation activates mast cells in the gastrointestinal mucosa and submucosa (Cho and Ogle, 1977; Gottwald et al., 1995; Santos et al., 1996). In addition, consistent association of mast cells and fibers containing either substance P or CGRP has been demonstrated in the rat mesentery (Stead et al., 1987; Crivellato et al., 1991). Therefore, mast cell-derived substances known to activate capsaicin-sensitive afferent fibers (Maggi, 1991; Akoev et al., 1996) may play an intermediary role in mediating the capsaicin and CGRP dependent gastric hyperemic response to i.c. RX 77368 at 1.5 ng. In the present study, we assessed the

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influence of the mast cell stabilizer, ketotifen (Grant et al., 1990) on the increase in gastric mucosal blood flow induced by RX 773368 injected i.c. at a low dose in urethane-anesthetized rats.

2. Material and methods

2.1. Animals

Male Sprague–Dawley rats (Harlan Laboratories, San Diego, CA) weighing 250–275 g were housed under conditions of controlled temperature (20 ± 3°C) and illumination (12 h light cycle starting at 6 a.m.). Rats were provided ad libitum Purina Laboratory Chow (Ralston, Purina, St. Louis, MO) and tap water. Food was withheld for a 24 h period before the experiments and free access to drinking water was maintained. All experiments were performed in rats anesthetized with urethane (1.25 g/kg, i.p.) under protocols reviewed and approved by the animal welfare committee at the VA West Los Angeles Research Center. Rectal temperature was maintained at 36–37°C throughout the duration of the study by a heating lamp placed above the animal.

2.2. Drugs

RX 77368, (*p*-Glu–His-(3,3′-dimethyl)-Pro-NH_2; Ferring Pharmaceuticals, Feltham, Middlesex) was stored in a stock solution (3 μg/10 μl, 0.1% bovine serum albumin/saline) at −70°C. The stock solution of RX 77368 was dissolved in 0.9% saline (pH = 7.0) before injection. Ketotifen (Sigma, St Louis, MO) was dissolved in 0.9% saline.

2.3. Surgeries

All the surgical procedures performed for simultaneous measurements of gastric mucosal blood flow, mean arterial pressure, and gastric acid secretion, as well as drug administration were essentially as previously described (Király et al., 1994; Király et al., 1997b).

2.3.1. Catheter into the cisterna magna

After the head was fixed in a Kopf stereotaxic instrument (Model 900), a small pin hole was made into the middle of the atlanto–occipital membrane with a 25 gauge needle, about 1–1.5 mm caudal to the edge of the occipital bone. A polyethylene catheter (PE-10, length: 8 cm; dead space: 5 μl) was inserted through the hole into the cisterna magna. The catheter was connected to a 50 μl Hamilton microliter syringe. Successful cannulation was verified by leakage of clear cerebrospinal fluid from the catheter. A drop of cyanoacrylate (Krazy Glue, Itasca, IL) was used to hold the catheter in position.

2.3.2. Cannulation of the trachea and vessels

After cannulation of the cisterna magna, a tracheotomy was performed to facilitate ventilation and enable the administration of hydrogen, and the esophagus was ligated. A PE-50 catheter was placed into the femoral artery for monitoring blood pressure with a Statham P23 Dd transducer. Another PE-50 catheter was inserted into the jugular vein and 0.9% saline was continuously infused at the rate of 1.5 ml/h through the jugular vein to maintain hydration and deliver drugs.

2.3.3. Gastric cannula

To monitor gastric acid secretion, the stomach was cannulated as follows. After a midline laparotomy, the stomach was exteriorized, the pylorus was ligated, and an incision was made into the forestomach to insert a double-lumen cannula (outer Tygon tube, 7 mm diameter; inner polyethylene catheter, 2 mm diameter), which was secured by ligature. Physiological saline (pH = 7.0) at room temperature was infused by the inner cannula at a rate of 0.5–0.7 ml/min. The effluent was collected continuously by flow drainage from the outer tube and separated in 15 min fractions.

2.3.4. Gastric electrode placement

After a small incision on the surface of the anterior wall of the stomach, a platinum needle electrode was inserted from the serosa into the basal portion of the gastric mucosa. The electrode was positioned into a high blood flow area, between the two branches of the left gastric artery, closer to the lesser than the greater curvature of the corpus of the stomach as previously described (Tanaka et al., 1993). The reference electrode (Ag–AgCl) was placed inside of the peritoneal cavity.

2.3.5. Simultaneous measurements of gastric mucosal flood flow, acid secretion and systemic blood pressure

After an equilibration period of 1 h, gastric mucosal blood flow was measured by the hydrogen gas-clearance technique as previously described and validated (Leung et al., 1984). Briefly, each measurement involved a 30 min period including 15 min of saturation with 3% hydrogen gas and 15 min desaturation of the tissue. The gas clearance was analyzed by a computerized monoexponential direct curve-fitting program (Livingston et al., 1989). Values are expressed in ml/min/100 g. The mean arterial blood pressure was recorded continuously throughout the duration of each experiment and expressed in mm Hg. Gastric mucosal vascular resistance was calculated by dividing the systemic blood pressure value at the beginning of the desaturation period by the respective gastric mucosal blood flow and expressed in mm Hg/ml/min/100 g. The systemic arterial blood pressure time point selected for the calculation of gastric mucosal vascular resistance was based on the rapid desaturation

during the first minutes after the 15 min saturation period which has a maximal influence on the final curve fit measuring blood flow (Livingston et al., 1989).

In the same preparation, the gastric effluent was continuously collected and separated into 15 min fractions which were titrated with 0.01 N NaOH using an automatic titrator (Radiometer, Copenhagen). Gastric acid output was expressed in μmol/30 min.

2.4. Experimental procedures

The gastric mucosal blood flow was measured twice before (basal) and once after administration of ketotifen (2 mg/kg i.v. bolus followed by an infusion of 2 mg/kg/h throughout the experiment) or vehicle (0.3 ml followed by 1.5 ml/h infusion throughout the experiment). Thirty minutes after the start of the intravenous infusion of ketotifen or vehicle, RX 77368 (1.5 ng) was injected i.c. in 10 μl in both vehicle- and ketotifen-pretreated groups and gastric mucosal blood flow was measured for the 60 min period post injection. Mean arterial pressure values were analyzed under basal conditions, at 15 min after i.v. infusion of vehicle or ketotifen and at 15 and 45 min after i.c. injection of RX 77368. The dose of TRH analogue was based on previous experiments showing an increase in gastric mucosal blood flow through vagal capsaicin- and CGRP-dependent pathways (Király et al., 1997b). The dose of ketotifen was based on a previous in vivo study showing mast cell stabilizing properties (Grant et al., 1990).

2.5. Statistics

Results are expressed as means ± S.E.M. Data were analyzed with ANOVA followed by Student–Newman–Keuls multiple comparisons test. MAP results before and after i.c. injection of RX 77368 within the same group were also analyzed by paired *t*-test. $P < 0.05$ was considered statistically significant.

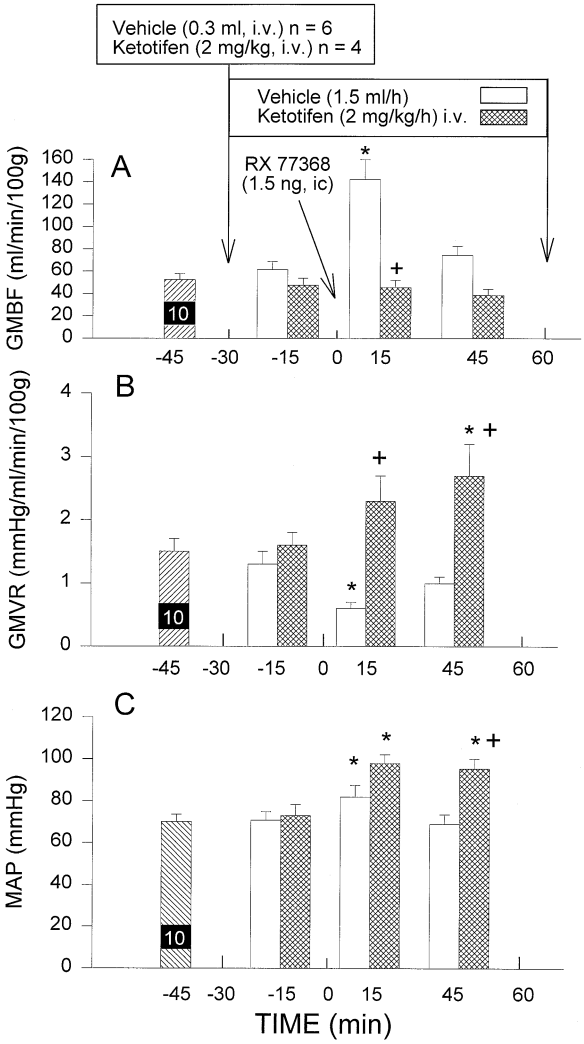

Fig. 1. Time course of the ketotifen effect on RX 77368 injected intracisternally (i.c.)-induced changes in gastric mucosal blood flow, GMBF, (A), gastric mucosal vascular resistance, GMVR, (B), and mean arterial pressure, MAP, (C) © in urethane-anesthetized rats. Each column represents the mean ± S.E.M. of 4–6 rats. * $P < 0.05$ compared with basal levels (−45 min); + $P < 0.05$ compared with vehicle plus RX 77368-treated group. GMBF and GMVR values represent the 15 min period from time indicated in min.

Table 1
Gastric acid secretion in response to i.c. injection of RX 77368 (1.5 ng) in ketotifen- or vehicle-treated urethane anesthetized rats

Treatment [a]	Gastric acid output (μmol/30 min) [b]			
	basal	time (min)		
		0	30	60
Vehicle	2.3 ± 0.5	4.3 ± 1.7	5.1 ± 1.5	3.0 ± 0.7
Ketotifen	2.1 ± 0.6	2.6 ± 0.7	3.5 ± 1.0	3.7 ± 0.9

[a] After basal measurements, two groups of rats were injected i.v. with vehicle (0.3 ml) or ketotifen (2 mg/kg) followed by i.v. infusion of vehicle (1.5 ml/h) or ketotifen (2 mg/kg/h) for 30 min before and 60 min after i.c. injection of RX 77368 (1.5 ng.). Acid secretion was monitored throughout the study and represents 30 min periods without treatment (basal) after vehicle or ketotifen treatment alone (0 time) and after i.c. injection of RX 77368 (30 and 60 min).

[b] Mean ± S.E.M. of 6 and 4 rats in vehicle and ketotifen groups respectively.

3. Results

Basal values of gastric mucosal blood flow were 52.4 ± 5.5 ml/min/100 g, mean arterial blood pressure, 70 ± 4 mm Hg, gastric mucosal vascular resistance, 1.5 ± 0.2 mm Hg/ml/min/100 g and gastric acid secretion, 2.2 ± 0.2 μmol/30 min in urethane anesthetized rats (Fig. 1, Table 1). Intravenous infusion of the vehicle for 15–30 min period did not modify these parameters (Fig. 1, Table 1). In vehicle-infused rats, RX 77368 injected i.c. at 1.5 ng

increased gastric mucosal blood flow to 141.8 ± 18.1 ml/min/100 g ($P < 0.05$) and decreased gastric mucosal vascular resistance by 59% (0.6 ± 0.1 mm Hg/ml/min/100 g, $P < 0.05$) as monitored during the 15–30 min period after TRH analog injection; at the 45–60 min period, these changes are returning to basal levels (Fig. 1A–B). Systemic blood pressure was increased to 82 ± 6 mm Hg ($P < 0.05$, paired-*t*-test) at 15 min after i.c. injection of RX 77368 and values were returned to basal levels at 45 min (Fig. 1C). There were no significant changes in gastric acid secretion induced by i.c. injection of RX 77368 at 1.5 ng during the 60 min period post injection (Table 1).

Ketotifen alone (2 mg/kg i.v. bolus, and 2 mg/kg/h, i.v. infusion) did not alter basal gastric mucosal blood flow, gastric vascular resistance, mean arterial pressure and gastric acid secretion as monitored during the first 15–30 min period (Fig. 1, Table 1). Ketotifen completely prevented i.c. RX 77368 at 1.5 ng-induced increase in gastric mucosal blood flow and values were maintained similar to basal level throughout the 60 min experimental period (Fig. 1A). The decrease in gastric mucosal vascular resistance induced by i.c. injection of RX 77368 at 1.5 ng was abolished by ketotifen and values (mm Hg/ml/min/100 g) were significantly increased (2.7 ± 0.5) at the 45–60 min period after RX 77368 injection compared with basal levels (Fig. 1B). In ketotifen-treated rats, the hypertensive response induced by i.c. injection of RX 77368 was further enhanced by 16 mm Hg at 15 min post injection ($P < 0.05$) and maintained significantly increased (96 ± 5 mm Hg) at 45 min post injection while the mean arterial pressure in vehicle-treated group was returned to basal levels (69 ± 5 mm Hg) (Fig. 1C). There was no change in gastric acid secretion in ketotifen-treated rats injected i.c. with 1.5 ng of RX 77368 (Table 1).

4. Discussion

The stable TRH analog, RX 77368, injected i.c. at 1.5 ng increased gastric mucosal blood flow and systemic blood pressure while gastric mucosal resistance was decreased during the 15–30 min post injection in vehicle-pretreated, urethane-anesthetized rats. By contrast, there were no changes in gastric acid secretion for the 30 min following the injection. The vascular changes induced by i.c. injection of RX 77368 at 1.5 ng are short lasting as shown by the return to basal levels at the 45–60 min period post RX 77368 injection (Király et al., 1997b; present data). Longer lasting vascular changes were observed after i.c. injection of RX 77368 at a higher dose (Tanaka et al., 1993; Király et al., 1994). The specificity of the TRH response was previously established by the lack of vascular responses upon i.c. injection of vehicle (Király et al., 1994; Király et al., 1997b).

Previous reports indicate that the gastric hyperemic response to i.c. injection of TRH or RX 77368 is vagal-cholinergic mediated (Thiefin et al., 1989; Király et al., 1997a; O-Lee et al., 1997). In the present study, ketotifen, administered at a dose which had no effect on basal vascular parameters, prevented i.c. RX 77368 at 1.5 ng induced-increase in gastric blood flow and decrease in gastric vascular resistance. Ketotifen is a well established mast cell stabilizer as shown by in vivo or in vitro studies in which gastrointestinal mast cell activation induced by various treatments (toxin A, calcium ionophore, nitric oxide synthase inhibitor, substance P, and *Helicobacter pylori*) was abolished by ketotifen (Hogaboam et al., 1993; Kubes et al., 1993; Pothoulakis et al., 1993; Grönbech and Lacy, 1994; Kurose et al., 1994). However, there is evidence in isolated intestinal smooth muscle preparations that ketotifen may also act as a non selective weak antimuscarinic receptor antagonist (Eltze et al., 1992) although such findings have not been confirmed (Abu-Dalu et al., 1996). It is unlikely that the ketotifen inhibitory action observed in the present study is related to its weak antimuscarinic properties (Eltze et al., 1992). The atropine-sensitive stimulation of other gastric functions (motility at 1.5 ng and acid secretion at 30 ng) induced by i.c. injection of RX 77368 is not modified by ketotifen under otherwise similar conditions (unpublished data). Likewise, the acid response to pylorus ligation is not altered by intragastric administration of 1–10 mg/kg of ketotifen (Karmeli et al., 1991; Okabe et al., 1992).

Although we did not investigate directly whether i.c. injection of RX 77368 at 1.5 ng induced a vagal cholinergic mast cell activation, convergent findings support this hypothesis. Electrical vagal stimulation decreased mast cell count in the gastric mucosa and submucosa through atropine-sensitive pathways in rats (Cho and Ogle, 1977; Cho and Ogle, 1979; Gottwald et al., 1992). Cold exposure, known to stimulate gastric vagal activity through excitation of medullary TRH neurons (Niida et al., 1991; Yang et al., 1994), results in a cholinergic mediated degranulation of gastric mast cells (Cho and Ogle, 1979). Likewise, RX 77368 injected i.c. induced a vagal muscarinic dependent increase in the intestinal release of protease II, (Santos et al., 1996) a specific marker for activated mucosal mast cells (Miller et al., 1989). Connective tissue mast cells were also shown to respond directly to a cholinergic receptor agonist (Fantozzi et al., 1978; Masini et al., 1985). In addition, the stomach contains a dense population of mast cells (Catto-Smith and Ripper, 1995) and a close anatomical relationship between mast cells, neurons and arteriolar walls has been observed in the gastric and intestinal submucosa (Newson et al., 1983; Johnson and Krenger, 1992; Grönbech and Lacy, 1994).

Mechanisms through which ketotifen prevents the gastric hyperemic response to i.c. injection of RX 77368 at 1.5 ng are still to be established. However, there is evidence supporting the view that ketotifen action may be mediated by preventing mast cells-induced activation of

capsaicin-sensitive afferent fibers. This concept is attractive since capsaicin-sensitive afferent fibers containing CGRP are closely apposed to mast cells (Stead et al., 1987; Stead et al., 1989; Crivellato et al., 1991). In addition, mast cell-derived substances such as serotonin, histamine, or prostaglandin E_2 (Johnson and Krenger, 1992) are capable of sensitizing or activating capsaicin-sensitive fibers which are ultimately reflected by the release of CGRP (Hua and Yaksh, 1993; Akoev et al., 1996). Moreover, we recently showed that capsaicin pretreatment or the CGRP receptor antagonism, CGRP-(8-37) injected i.v. completely abolished i.c. RX 77368 at 1.5 ng induced gastric hyperemia (Király et al., 1997b). We also obtained preliminary electrophysiological evidence that i.c. injection of RX 77368 at 1.5 ng induces an atropine-sensitive activation of gastric splanchnic afferent fibers which is alleviated by ketotifen (O-Lee et al., 1996). Taken together, these data suggest that the cholinergic dependent gastric hyperemia to central TRH, injected at a dose subthreshold to induce an acid secretory response, could be brought about by an interaction between vagal cholinergic stimulation and gastric mast cell response activating efferent function of capsaicin-sensitive afferent fibers to release the vasodilatory peptide, CRRP.

Central injection of TRH or TRH analog increases systemic blood pressure which is dependent upon sympathetic nervous system activation (Mattila and Bunag, 1986; Sirén et al., 1988; Thiefin et al., 1989; Király et al., 1997a). However, while central injection of TRH stimulates splanchnic nerve activity in the adrenal and renal branches, there is a decrease in the activity in the gastric branch (Brown, 1981; Somiya and Tonoue, 1984; Mattila and Bunag, 1986). In the present study, i.c. injection of RX 77368 at 1.5 ng induces a short lasting rise (11 mm Hg) in systemic blood pressure as previously reported using a similar dose of RX 77368 (Király et al., 1997b). The decrease in gastric mucosal resistance in the presence of an increase in total systemic vascular resistance indicates a local vasodilation of gastric mucosal arterioles induced by i.c. RX 77368 at 1.5 ng. In ketotifen-treated rats, the rise in mean arterial pressure induced by i.c. injection of RX 77368 was of higher magnitude (25 mm Hg) and longer duration (over 45 min) and there was an increase instead of a decrease in gastric mucosal vascular resistance. Since ketotifen did not influence basal mean arterial pressure, these results indicate that mast cells alleviate the systemic hypertensive response to i.c. RX 77368 at 1.5 ng most likely through the release of vasodilatory transmitters (Hannon et al., 1995). Ketotifen induced-blockade of gastric hyperemia is unlikely to be the consequence of the rise in systemic arterial blood pressure. Central injection of TRH or RX 77368 at higher doses induces similar hypertensive responses (28–30 mm Hg) while the gastric blood flow is increased above 100% and vascular resistance is decreased (Thiefin et al., 1989; Király et al., 1994; Király et al., 1997a). In addition, atropine, vagotomy, capsaicin or CGRP receptor antagonist pretreatments abolished central TRH or RX 77368 induced increase in blood flow and decrease in vascular resistance in the gastric mucosa while the rise in systemic blood pressure was not inhibited (Thiefin et al., 1989; Király et al., 1994; Király et al., 1997a; Király et al., 1997b). The increase in gastric vascular resistance observed after RX 77368 in rats pretreated with ketotifen (present study), capsaicin and CGRP receptor antagonist (Király et al., 1997b) may reflect gastric vasoconstriction when local vasodilatory mechanisms are blocked by these treatments due to catecholaminergic influence as observed in other vascular beds after central injection of TRH (Sirén et al., 1988).

In summary, stabilization of mast cells by ketotifen abolished the gastric hyperemic response to TRH analog injected i.c. at a dose subthreshold to stimulate gastric acid secretion. These findings suggest a significant role of mast cells in the regulation of gastric mucosal blood flow in response to a low level of central vagal activation. These observations also support the notion that mast cells, in addition to their important role in immunological and pathologic processes in the gastrointestinal tract (Crowe and Perdue, 1992), may participate in the physiological vagal regulation of gastric mucosal blood flow. As different stimuli may evoke different patterns of mast cell mediator release (Theoharides et al., 1985), further studies are required to delineate the mast cell mediators involved as well as the exact mechanisms underlying the interaction between mast cells and capsaicin-sensitive afferent fibers in response to intracisternal injection of the stable TRH analog at low dose.

Acknowledgements

Supported by the National Institute of Mental Health, Grant MH-00663 and the National Institute of Diabetes and Digestive and Kidney Diseases, Grants DK-30110 and DK-41301 (Animal and Blood Flow Cores). We thank Dr. T. Waters (Ferring Pharmaceuticals, Feltham, Middlesex) for the supply of RX 77368. Paul Kirsch is acknowledged for his help in the preparation of the manuscript.

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