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SR120819A, an orally-active and selective neuropeptide Y Y1 receptor antagonist

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Abstract An orally-active antagonist of neuropeptide Y (NPY) Y_1 receptors, SR 120819A, has been characterized. This compound displays highly selective and competitive affinity for rat, guinea-pig and human (K_i = 15 nM) NPY Y_1 receptors. In vitro, SR 120819A blocks the inhibitory effect of NPY on adenylyl cyclase activity in human SK-N-MC cells and that of the selective Y_1 agonist, [Leu^{31},Pro^{34}]NPY, on rabbit vas deferens contraction (pA_2 = 7.20 ± 0.07). In vivo, by intravenous route, this compound acts as an antagonist in anesthetized guinea-pigs and, notably, after oral administration, SR 120819A counteracts the pressor response of [Leu^{31},Pro^{34}]NPY (5 μg/kg i.v.) with a long duration of action (>4 h at 5 mg/kg p.o.). Thus, SR 120819A is the first orally-effective NPY Y_1 receptor antagonist yet described. It could be a useful tool for exploring the role of NPY and the therapeutic relevance of an antagonist at NPY Y_1 receptors.

Key words: Neuropeptide Y; NPY; Y_1 receptor antagonist; SR 120819A

1. Introduction

Neuropeptide Y (NPY) is a 36 amino-acid regulatory peptide abundantly found in the central and peripheral nervous system of mammals [1]. Related to its widespread distribution, NPY exerts a variety of biological effects, especially on cardiovascular regulation, food intake, metabolism, behavioral and endocrine functions via three proposed receptor subtypes, Y_1, Y_2 and Y_3 [2]. In particular, through post synaptic Y_1 receptors, NPY and the related peptide, PYY, have potent vasoconstrictor effects on crucial territories such as coronary and cerebral vessels [3–5]. Among various central effects, the Y_1 receptor subtype also seems to be involved in the powerful stimulation of food intake by NPY [6].

Several lines of evidence have underlined the potential role of NPY in varying pathological states such as in some forms of hypertension, vasospasm, myocardial infarction, obesity and boulimia [7,8]. Accordingly, the search for selective, orally-active NPY antagonists appears essential both for further investigating the role of NPY and for designing a new class of potential therapeutical agents. Until now, however, no peptide or non-peptide antagonist with reasonable affinity or selectivity at NPY receptors was available (for review see [9]). In this field, we recently reported the design of the first potent, selective and orally-effective NPY Y_1 receptor antagonists yet described [10]. An antagonist of NPY Y_1 receptors active in vitro and in vivo by intravenous route has also been recently claimed [11]. Based on the known interactions of NPY with Y_1 receptors [12–14], we designed SR 120819A (1-[2-[2-(2-naphtylsulfamoyl)-3-phenylpropionamido]-3-[4-[*N*-[4-(dimethylaminomethyl)-*cis*-cyclohexylmethyl]amidino]phenyl]propionyl]pyrrolidine, (R,R) stereoisomer) (Fig. 1), a member of an original chemical series of selective and orally-active NPY Y_1 antagonists. In the present study, we characterize the biochemical and pharmacological profile of this compound. Since marked species differences have been reported for several peptide ligand receptors [15] and in the amino acid sequence of cloned Y_1 receptors [16,17], special attention was paid to the evaluation of SR 120819A at human NPY Y_1 receptors in binding and functional studies, using the human neuroblastoma cell line SK-N-MC which only expresses Y_1 receptors.

2. Materials and methods

2.1. Materials

Chemicals. SR 120819A (1-[2-[2-(2-naphtylsulfamoyl)-3-phenylpropionamido]-3-[4-[*N*-[4-(dimethylaminomethyl)-*cis*-cyclohexylmethyl]amidino]phenyl]propionyl]-pyrrolidine, (R,R) stereoisomer) (Fig. 1) was synthesized in Sanofi Recherche (Toulouse, France). This compound was dissolved either in distilled water at 10^{-2} M or in the appropriate test solvent. NPY, [Leu^{31},Pro^{34}]NPY and bacitracin were from Sigma Chemical Co. (L'isle d'Abeau, France). Bovine serum albumin was obtained from Biosepra (Paris, France). Tissue culture reagents were from Boehringer-Mannheim (Meylan, France). EDTA, HEPES were purchased from Merck-Clevenot (Nogent sur Marne, France). All other chemicals were from Prolabo (Paris, France). The radioligands, ^{125}I-NPY and ^{125}I-PYY (2,000 Ci/mmol), were from Amersham and New England Nuclear (Les Ulis, France), respectively, and cAMP assay kits were obtained from Amersham.

Biological materials. Male Wistar rats (250–350 g) were from Iffa-Credo (Lyon, France). Male New Zealand rabbits (2.5–3 kg) from Gendre (Lusignan, France) were used for in vitro isolated preparations, and Hartley guinea-pigs (300–400 g) were from Charles River (France). SK-N-MC cells, isolated from a human metastatic neuroblastoma, were purchased from the American Type Culture Collection (ATCC, HTB 10) (Rockville, MD). Frozen human cortex was obtained from the International Institute for the Advancement of Medicine, the Key Stone Skin Bank (Exton, USA).

2.2. Cell culture

SK-N-MC cells, plated in 175 cm^2 culture flasks, were grown in minimal essential medium (MEM) with Earle's salts supplemented with 10% fetal calf serum, 1% nonessential amino acids, 1 mM sodium pyruvate, 1% vitamins, 4 mM glutamine, penicillin-G (100 IU/ml) and

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streptomycin (100 μg/ml) in a 95% air, 5% CO_2 humidified atmosphere at 37°C. Culture medium was removed every other day and SK-N-MC cells were subcultured by treatment with 0.05% trypsin, 0.02% EDTA.

2.3. Membrane preparations

Rat, guinea-pig and human cortex plasma membranes (P_2 fraction) were obtained by the method of Unden [18] and stored as aliquots in liquid nitrogen until required. SK-N-MC membranes from confluent cells were prepared as previously described [19]. Protein concentration was determined by the method of Bradford using bovine serum albumin as a standard [20].

2.4. Binding assays

In binding experiments, membranes (0.1 mg/ml) from rat, guinea-pig or human cortex, or from SK-N-MC cells were incubated for 60 min at 30°C in a Krebs–Ringer buffer (pH 7.4) containing 20 mM HEPES, 1% bovine serum albumin, 0.25 mg/ml bacitracin and 0.1 nM ^{125}I-NPY or ^{125}I-PYY. The reaction was stopped by rapid filtration through Whatman GF/C filters, washed with 3 × 4 ml of ice-cold buffer and the radioactivity, bound to the filters, was measured by scintillation counting. Non-specific binding was determined in the presence of 0.3 μM porcine NPY. All experiments were carried out in duplicate. Data from binding experiments were analyzed using a non-linear regression program [21] and inhibition constants (K_i) value calculated according to Cheng and Prusoff equation [22].

For selectivity, binding assays were carried out using standard protocols [23].

2.5. Adenylyl cyclase assays

SK-N-MC cells, cultured in 24-well plates, were incubated for 10 min in a 2.5 mM HEPES/Tris medium (pH 7.5) containing 140 mM NaCl, 5 mM KCl, 1.8 mM $CaCl_2$, 0.2% bovine serum albumin, 4.5 g/l glucose and 1 mM isobuthyl-methyl-xanthine. Different concentrations of SR 120819A (or vehicle) were added 1 min prior to the addition of 10 nM NPY. 1 min later, 0.5 μM forskolin was added and the incubation was performed 20 min. The reaction was stopped by aspiration of the medium and rapid addition of 1 ml methanol. After extraction, cAMP content was measured using cAMP assay kits.

2.6. Isolated rabbit vas deferens preparations

The prostatic segments of male New Zealand rabbits' vas deferentia were studied as previously described [24]. Electrical field stimulation consisted of rectangular bipolar pulses of 1 ms duration and of 0.1 Hz frequency, applied at a submaximal voltage. The antagonist effect of SR 120819A on [Leu^{31},Pro^{34}]NPY-induced twitch contraction inhibition was expressed by a pA_2 value calculated according to Van Rossum's method [25].

2.7. Arterial blood pressure in anesthetized guinea pigs in vivo

Guinea-pigs were anesthetized with sodium pentobarbital (30 mg/kg i.p.) and assays performed as in [26]. Diastolic blood pressure was measured using a Statham P10EZ pressure transducer coupled to a RS 3400 polygraph (Gould). SR 120819A was injected intravenously (0.1–1 mg/kg) into anesthetized animals, or orally (1 to 10 mg/kg) 50 min prior to anesthesia, and tested on 5 μg/kg i.v. [Leu^{31},Pro^{34}]NPY-induced hypertension which was injected at different times. All values are given as means ± S.E.M. and statistical analysis of data was performed with Anovarep followed by a Dunnet's *t*-test for multiple comparisons. The level of significance was taken as $*P < 0.05$.

3. Results

3.1. Interaction of SR 120819A with rat, guinea-pig and human NPY Y_1 receptors and selectivity profile

SR 120819A (Fig. 1), inhibited in a concentration-dependent manner ^{125}I-NPY and ^{125}I-PYY specific binding to NPY Y_1 receptors in rat and guinea-pig cortex, and in the human neuroblastoma SK-N-MC cell line. Hill coefficient values were close to unity compatible with a single-site competitive model. It is important to note that, under similar experimental conditions, K_i (inhibition constant) values were in the 10 nanomolar range and were similar in the three species, including man (Table 1).

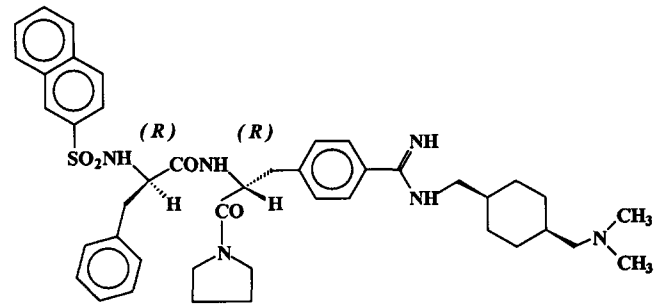

Fig. 1. Chemical structure of SR 120819A.

Saturation binding experiments were performed on human SK-N-MC preparation, in the presence or absence of SR 120819A (15 and 30 nM). Scatchard analysis of data demonstrated that SR 120819A interacted with human Y_1 receptors in a fully competitive manner since in the presence of this molecule the apparent dissociation constant (K_d) was significantly changed, whereas the maximal binding capacity (B_{max}) was not modified (Fig. 2A). The K_i value calculated from Scatchard plots (K_i = 15 nM) was in good agreement with that obtained according to the Cheng and Prusoff equation [22] from competition experiments (K_i = 17 ± 6 nM).

The selectivity of SR 120819A for NPY Y_1 receptors was evaluated by measuring the ability of this compound to interact with NPY Y_2 or Y_3 receptors. As shown in Table 1, SR 120819A was highly selective, since at 10 μM it did not bind to human cortex Y_2 receptors. In addition, it did not inhibit the functional contractile response of NPY in the Y_3 rat colon model [27] in concentrations of up to 1 μM (5 ± 2% inhibition at 1 μM). Moreover, in thirty classical binding assays, SR 120819A, 1 μM, did not interact with receptors of non-peptide or peptide ligands (data not shown).

3.2. Effect of SR 120819A on adenylyl cyclase activity in SK-NM-C cells

The signalling mechanisms, linked to the NPY Y_1 receptor subtype, involved Ca^{2+} mobilization and adenylyl cyclase inhibition, and both mechanisms have been observed in SK-N-MC

Table 1
Affinity of SR 120819A for NPY Y_1 and Y_2 receptors

	Y_1			Y_2
	K_i (nM)			Inhibition (%) at 10 μM
Tissues	Rat cortex	Guinea-pig cortex	Human SK-N-MC cells	Human cortex
Ligands				
^{125}I-NPY	15 ± 6	22 ± 6	21 ± 8	0
^{125}I-PYY	11 ± 3	20 ± 5	17 ± 6	0

Binding assays were performed as described in section 2.4 using 100 pM ^{125}I-NPY or ^{125}I-PYY, 20 μg protein membranes and increasing concentrations of SR 120819A (10^{-10} to 10^{-5} M). Inhibition constants (K_i) were determined from competition experiments calculated according to Cheng and Prusoff's equation [22]. Values are given as the mean ± S.D. of 3 to 6 independent determinations performed in duplicate.

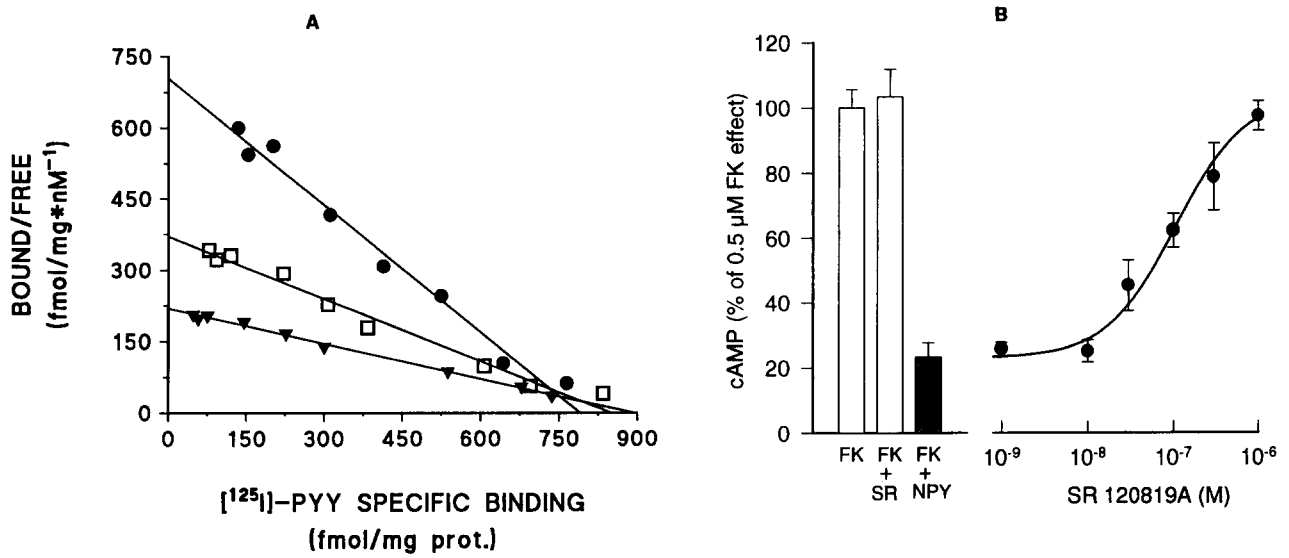

Fig. 2. Binding (A) and adenylyl cyclase activity (B) of SR 120819A at human NPY Y_1 receptors in SK-N-MC cells. (A) Scatchard plots of ^{125}I-PYY binding to human SK-N-MC membranes without (●) or with 15 (□) and 30 (▼) nM SR 120819A. The radioligand (100 pM) was incubated alone or in the presence of increasing concentrations of PYY with membranes (20 µg protein) for 1 h at 30°C. Each point is the mean calculated from a typical experiment performed in duplicate. (B) SR 120819A antagonism on 10 nM NPY-induced cAMP inhibition in SK-NM-C cells. Histograms represent the effect of 0.5 µM forskolin (FK) alone or in the presence of either 1 µM SR 120819A or 10 nM NPY. The results are expressed as a percent of maximal 0.5 µM forskolin (FK) effect. Each point is the mean ± S.E.M. of 3 independent experiments performed in triplicate.

cells [28]. In order to determine the agonist or antagonist properties of SR 120819A, we examined the activity of this compound on NPY-induced inhibition of 0.5 µM forskolin-evoked cAMP accumulation in SK-N-MC cells, in vitro. As shown in Fig. 2B, SR 120819A by itself did not affect the cAMP level significantly at 1 µM, but dose-dependently antagonized NPY inhibition of cAMP accumulation with an IC_{50} value (with 95% confidence interval) of 92 nM (66–140) (n = 3).

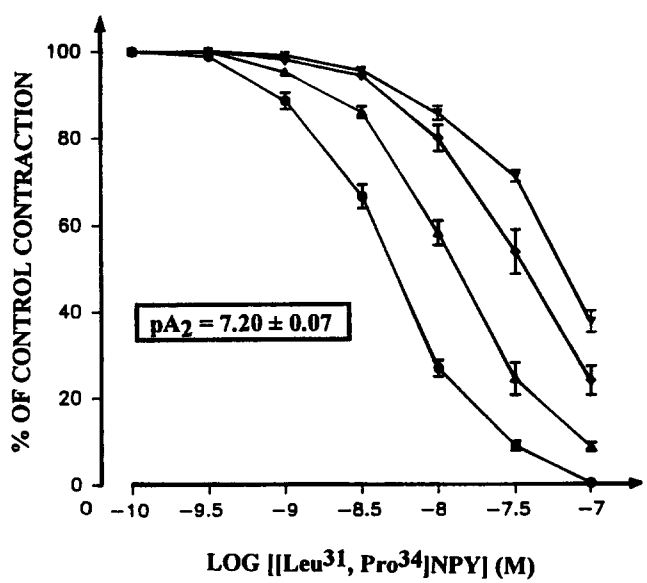

Fig. 3. Effect of SR 120819A on the electrically-stimulated rabbit vas deferens. Cumulative concentration–response curves for [Leu^{31},Pro^{34}]NPY on the amplitude of twitch contractions elicited by electrical field stimulation were established in the absence (●) or in the presence of 0.1 (▲), 0.3 (◆) and 1 (▼) µM of SR 120819A. Results are expressed as percentage of the twitch control responses measured after 45 min incubation with or without SR 120819A and are the mean ± S.E.M. of 5 to 6 experiments.

3.3. *Effect of SR 120819A on the electrically-stimulated rabbit vas deferens*

In vitro, in the Y_1 isolated rabbit vas deferens model, the selective Y_1 receptor agonist [Leu^{31},Pro^{34}]NPY, induced rapid, concentration-dependent inhibition of the twitch contractions with a pD_2 value of 8.32 ± 0.03 (n = 6) consistent with what has been already published [24]. SR 120819A (0.3 to 1 µM) produced a parallel rightward shift in the [Leu^{31},Pro^{34}]NPY concentration–response curve without significantly affecting the maximal contractile response (Fig. 3). Schild analysis of these data yielded a pA_2 value of 7.20 ± 0.07 with a slope about one, indicating competitive antagonism against [Leu^{31},Pro^{34}]NPY. In addition, SR 120819A exhibited no NPY agonist activity in modifying the electrically-induced twitch contraction in concentrations of up to 10 µM, when tested alone.

3.4. *Effect of SR 120819A in anesthetized guinea-pigs in vivo*

In anesthetized guinea-pigs, 5 µg/kg of [Leu^{31},Pro^{34}]NPY i.v. induced a rise in diastolic blood pressure of approximately 20 mmHg. No tachyphylaxis occurred in this preparation after repeated injections separated by 30 min (Fig. 4). Bolus intravenous injection of SR 120819A (0.1 to 1 mg/kg) caused dose-dependent inhibition of the pressor response to exogenous [Leu^{31},Pro^{34}]NPY (5 µg/kg i.v.). The inhibitory effect of SR 120819A lasted significantly for 3 h at the highest dose (1 mg/kg) (Fig. 4A). When administered orally, SR 120819A (1 to 10 mg/kg) also inhibited the Y_1 agonist hypertensive response in a concentration-dependent manner. The inhibitory effect was significant at the two highest doses of 5 and 10 mg/kg and was maintained for at least 4 h, demonstrating a long lasting oral effect of SR 120819A (Fig. 4B). It is important to underline that no significant intrinsic agonistic effect was observed with

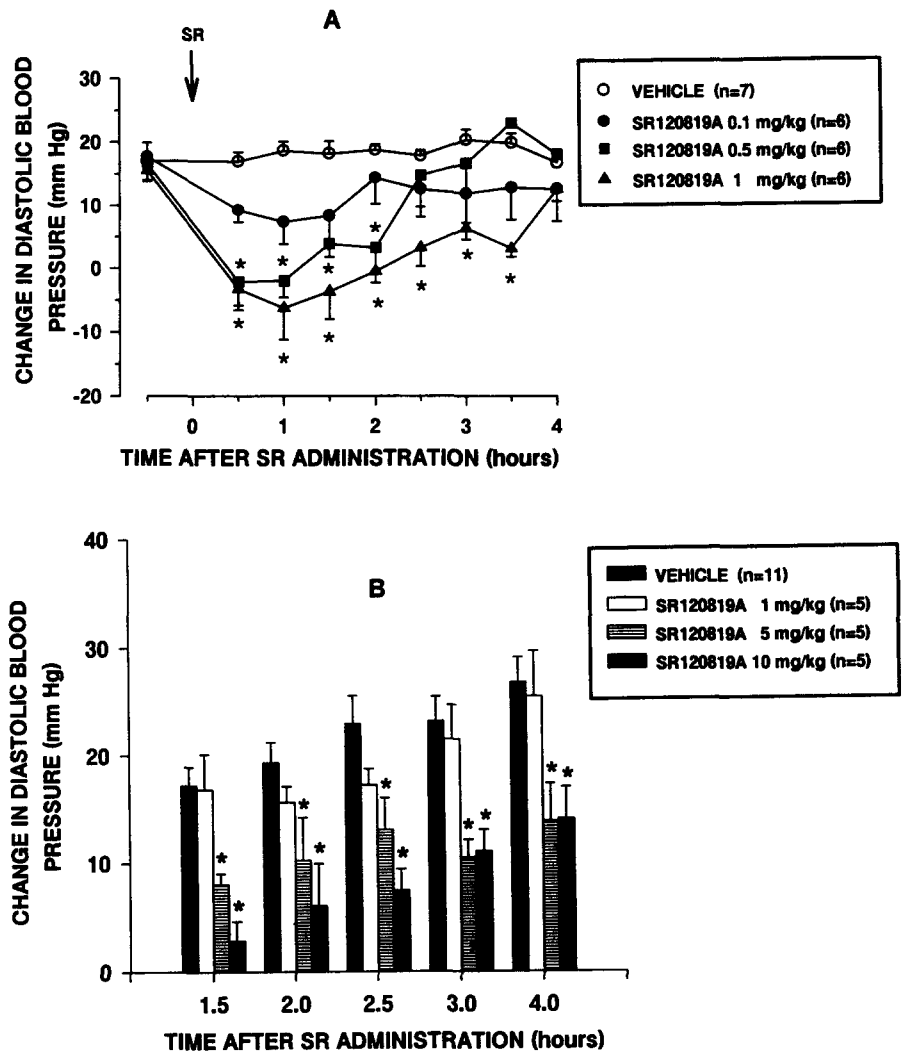

Fig. 4. Time-course of the inhibitory effect of SR 120819A intravenously (A) and per os (B) on [Leu^{31},Pro^{34}]NPY (5 μg/kg i.v.)-induced hypertension in anesthetized guinea-pigs. Data are expressed as a change in diastolic blood pressure, observed after each [Leu^{31},Pro^{34}]NPY injection and represent the mean ± S.E.M. of 5 to 11 determinations per group. Statistical analysis was performed using Anovarep followed by Dunnett's test and the level of significance was taken as $^{*}P < 0.05$.

SR 120819A after intravenous or oral administration (not shown).

4. Discussion

The present study indicates that SR 120819A is a potent and selective ligand of NPY Y_1 receptors and displays both in vitro and in vivo antagonistic properties against NPY or [Leu^{31},Pro^{34}]NPY response. Moreover, SR 120819A is the first orally-active NPY Y_1 receptor antagonist, yet described.

So far, three NPY receptor subtypes have been identified and mediate the diverse biological effects described for this peptide [2]. The NPY Y_1 receptor subtype clearly supports the powerful pressor and vasoconstrictor action of NPY and seems also to be involved in several of its behavioural effects [3–6]. Furthermore, because NPY might be intimately implicated in several diseases (hypertension, heart failure, vasospasm [7–9]), the development of potent and specific NPY antagonists with, in addition, good oral bioavailability, may offer novel therapeutical approaches. Since in this field no compounds with such a profile are yet available, SR 120819A represents an important step forward in this research.

This compound displays high, selective affinity for NPY Y_1 receptors in vitro: in our binding studies, SR 120819A had similar affinity for NPY Y_1 receptors in different animal species (rat, guinea-pig) and, in particular, in the human neuroblastoma cell line, SK-N-MC (K_i = 15 nM). In this latter model, it has been shown that SR 120819A interacts in a fully competitive manner at NPY Y_1 receptors.

It is also important to underline the highly selective profile of this compound for Y_1 receptors versus other NPY receptor subtypes (Y_2 and Y_3) and various receptors in general as evidenced in several binding tests in vitro.

In addition, several functional studies in vitro and in vivo have been performed to address the antagonist or agonist properties of SR 120819A. The human neuroblastoma cell line, SK-N-MC, expressing selectively the functional Y_1 receptor subtype has provided an essential model for investigating both

the interaction and the subsequent activity of SR 120819A at NPY Y_1 receptors from human origin. Moreover, the two signalling mechanisms, i.e. Ca^{2+} mobilization and adenylyl cyclase inhibition, linked to NPY Y_1 receptor activation, have been demonstrated in SK-N-MC cells [28]. Studied in this model, SR 120819A dose-dependently inhibited the second messenger response elicited by NPY on adenylyl cyclase activity. Furthermore, we observed a good correlation between the binding affinity of SR 120819A to SK-N-MC Y_1 receptors and the antagonism on the physiological response of NPY on cAMP accumulation. Other results, in the electrically-stimulated rabbit vas deferens, previously characterized as a Y_1 model [24], confirmed the full competitive antagonist profile of SR 120819A in vitro ($pA_2 = 7.20 \pm 0.07$).

As NPY is one of the most potent contractile agents described so far and could be involved in the control of blood pressure in normal or even pathological situations via Y_1 receptors [3–5], the effect of SR 120819A on arterial blood pressure was investigated in anesthetized guinea-pigs. In this model, SR 120819A has shown considerable effect in antagonizing the hypertensive effect of [Leu^{31},Pro^{34}]NPY both by intravenous and oral routes. A remarkably long-lasting oral effect (>4 h at 5 mg/kg) together with the absence of agonist activity were observed.

Taken together, all these observations allow us to conclude that SR 120819A has a selective, orally-active Y_1 receptor antagonist profile. Therefore, this compound may be a promising tool for further studying the role of NPY, its receptor function, and also for exploring the therapeutic relevance of a NPY Y_1 receptor antagonist in various disorders.

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References

[1] Tatemoto, K. (1982) Proc. Natl. Acad. Sci. USA 79, 5485–5489.
[2] Grundemar, L. and Hakanson, R. (1994) Trends Pharmacol. Sci. 15, 153–159.
[3] Edvinsson, L., Hakanson, R., Wahlestedt, C. and Uddman, R. (1987) Trends Pharmacol. Sci. 8, 231–235.
[4] Potter, E.K. (1988) Pharmacol. Ther. 37, 251–273.
[5] Clarke, J.G., Kerwin, R., Larkin, S., Lee, Y., Yacoub, M., Davies, G.J., Hackett, D., Dawbarn, D., Bloom, S.R. and Maseri, A. (1987) Lancet, 1057–1059.
[6] Stanley, B.G. (1993) in: The Biology of Neuropeptide Y and Related Peptides (Colmers, W.F. and Wahlestedt, C. Eds.) pp. 457–509, Humana Press.
[7] Wahlestedt, C. and Reis, D.J. (1993) Annu. Rev. Pharmacol. Toxicol. 32, 309–352.
[8] Michel, M.C. and Buschauer, A. (1992) Drugs Fut. 17, 39–45.
[9] Selbie, L.A. and Tseng, A. (1994) Exp. Opin. Ther. Patents 4, 803–811.
[10] Serradeil-Le Gal, C., Valette, G., Rouby, P.E., Pellet, A., Villanova, G., Foulon, L., Lespy, L., Neliat, G., Chambon, J.P., Maffrand, J.P. and Le Fur, G. (1994) Soc. Neurosci. Abstr. 20, 907.
[11] Rudolf, K., Eberlein, W., Engel, W., Wieland, H.A., Willim, K.D., Wienen, W., Beck-Sickinger, A.G. and Doods, H.N. (1994) Eur. J. Pharmacol. 271, R11–R13.
[12] Allen, J., Novotny, J., Martin, J. and Heinrich, G. (1987) Proc. Natl. Acad. Sci. USA 84, 2532–2536.
[13] Kirby, D.A., Boublik, J.H. and Rivier, J.E. (1993) J. Med. Chem. 36, 3802–3808.
[14] Walker, P., Munoz, M., Martinez, R. and Peitsch, M.C. (1994) J. Biol. Chem. 269, 2863–2869.
[15] Serradeil-Le Gal, C., Raufaste, D., Marty, E., Garcia, C., Maffrand, J.P. and Le Fur, G. (1994) Biochem. Biophys. Res. Commun. 199, 353–360.
[16] Herzog, H., Hort, Y.J., Ball, H.J., Hayes, G., Shine, J. and Selbie, L.A. (1992) Proc. Natl. Acad. Sci. USA 89, 5794–5798.
[17] Larhammar, D., Blomqvist, G.A., Yee, F., Jazin, E., Yoo, H. and Wahlestedt, C. (1992) J. Biol. Chem. 267, 10935–10938.
[18] Unden, A., Tatemoto, K., Mutt, V. and Bartfai, T. (1984) Eur. J. Biochem. 145, 525–530.
[19] Serradeil-le Gal, C., Bourrie, B., Raufaste, D., Carayon, P., Garcia, C., Maffrand, J.P., Le Fur, G. and Casellas, P. (1994) Biochem. Pharmacol. 47, 633–641.
[20] Bradford, M.M., Anal. Biochem. 72, 248–254.
[21] Munson, P.V. and Rodbard, D. (1980) Anal. Biochem. 107, 220–239.
[22] Cheng, Y. and Prusoff, W. (1973) Biochem. Pharmacol. 22, 3099–3108.
[23] Cazaubon, C., Gougat, J., Bousquet, F., Guiraudou, P., Gayraud, R., Lacour, C., Roccon, A., Galingo, G., Barthelemy, G., Gautret, B., Bernhart, C., Perreaut, P., Breliere, J.C., Le Fur, G. and Nisato, D. (1993) J. Pharmacol. Exp. Ther. 265, 826–834.
[24] Doods, H.N. and Krause, J. (1991) Eur. J. Pharmacol. 204, 101–103.
[25] Van Rossum, J.M. (1963) Arch. Int. Pharmacodyn. Ther. 143, 299–330.
[26] Serradeil-Le Gal, C., Wagnon, J., Garcia, C., Lacour, C., Guiraudou, P., Christophe, B., Villanova, G., Nisato, D., Maffrand, J.P., Le Fur, G., Guillon, G., Cantau, B., Barberis, C., Trueba, M., Ala, Y. and Jard, S. (1993) J. Clin. Invest. 92, 224–231.
[27] Dumont, Y., Satoh, H., Cadieux, A., Taoudi-Benchekroun, M., Pheng, L.H., St-Pierre, S., Fournier, A. and Quirion, R. (1993) Eur. J. Pharmacol. 238, 37–45.
[28] Feth, F., Rascher, W. and Michel, M.C. (1991) Naunyn-Schmiedeberg's Arch. Pharmacol. 344, 1–7.