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A POTENT NEW β_2-ADRENOCEPTOR BLOCKING AGENT

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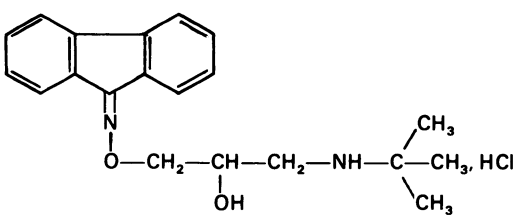
1 (*t*-Butyl-amino-3-ol-2-propyl) oximino-9 fluorene is a new β_2-adrenoceptor blocking agent with a pA_2 of 9.23 ± 0.25 on isolated trachea.

2 It provokes hypertension in normotensive rats and does not prevent arterial hypertension in SHR rats, although it does prevent the renin secretion normally induced by isoprenaline infusion.

Introduction

The β-adrenoceptor blocking agents normally used in therapy are characterized by an aminopropanolic side-chain, fixed on phenolic oxygen.

We synthesized a vinylogous series of β-adrenoceptor blocking agents where the side-chain is carried by an oximic oxygen atom. One of the compounds in this series, IPS 339, hydrochloride of (*t*-butyl-amino-3-ol-2-propyl) oximino-9 fluorene proved interesting.



IPS 339

Synthesis of IPS 339

Fluorenone-oxime was prepared by refluxing overnight 2 equivalents of hydroxylamine hydrochloride with fluorenone in dry pyridine. The sodium salt of the oxime was then reacted in dry dimethylformamide with epichlorhydrin, and the crude epoxide was treated with an excess of tertiobutylamine in ethanol to obtain IPS 339, a yellow powder scarcely soluble in water. It was converted to soluble hydrochloride (m.p. = 160°C) by passing HCl gas through an isopropanolic solution of IPS 339.

Chemical structure and purity were confirmed by elemental analysis; i.r., u.v., n.m.r. analysis and mass spectrometry (Mann, 1975).

Methods

pA_2 values

In vitro *experiments.* Guinea-pigs of either sex weighing between 350 and 550 g, were killed with a sharp blow on the head. Their atria and trachea were quickly excised and excess tissue was removed.

Isolated atria (Horii, Kawada, Takeda & Imai, 1974). Atria were divided carefully into right and left halves. The right atrium, which retained spontaneous rhythm, was used to assess the chronotropic action of the drugs. The initial resting tension was set at 0.5 gram. The left atrium, kept at a tension of 0.5 g, was stimulated electrically with square-wave pulses at a frequency of 2.5 Hz with voltages approximately 30% above the threshold (duration 2 milliseconds). The Krebs-Henseleit bathing solution, was kept at 32 ± 1°C, and aerated with a mixture of 95% O_2 and 5% CO_2. The antagonist was added to the bath 30 min before the assay.

Isolated trachea (Levy & Wilkenfeld, 1970). The trachea was cut spirally with a sharp scalpel to produce a thin strip of tracheal tissue. This spiral was cut into two equal segments and both were placed in a bath of Krebs-Henseleit solution. The perfusion fluid contained ascorbic acid (0.1 mg/ml) and phentolamine (0.1 µg/ml). Temperature was maintained at 37°C and the solution was gassed with 95% O_2 and 5% CO_2. An initial basal tension of 2.5 g was applied to each tracheal strip and the tissue was allowed to stand for 30 min before use. A constant level of tone was maintained by adding carbachol, at a concentration of 0.1 µg/ml to the bath. The carbachol was allowed to act for 15 minutes. Then, without washing, cumulative dose-response curves to isoproterenol were

determined before and after treatment with the antagonists. The assay of the antagonist started 60 min after adding it to the bath.

In vivo *experiments*. In all experiments, the antagonists were administered 30 min before isoprenaline.

Experiments with guinea-pigs. The animals (400–450 g) were anaesthetized with sodium pentobarbitone (70 mg/kg i.v.).

Resistance to lung inflation (Vargaftig & Coignet, 1969; Konzett & Rössler, 1940) was measured as follows: bronchoconstriction was induced with intravenous 5-hydroxytryptamine (5-HT) administered at 5 min intervals, in doses varying between 1 and 5 μg (but constant for each animal). When a group of three identical responses to 5-HT had been obtained, isoprenaline was administered intravenously and was followed by 5-HT again after 30 seconds. Isoprenaline reduced the bronchial response to 5-HT; doses varying between 0.05 and 0.5 μg were chosen to block 90–95% of the bronchoconstriction. Effective β-adrenoceptor blocking agents inhibited the antagonism of isoprenaline to 5-HT, and bronchoconstriction, which had previously been blocked, was again present. The percentage of bronchoconstrictor recovery was calculated by comparison of the response to 5-HT before and after administering β-blocking agents.

Cardiac rhythm was calculated from the blood pressure curve provided by a pressure transducer connected to the carotid artery.

Experiments with dogs. Mongrel dogs of either sex, ranging in weight between 8 and 20 kg were premedicated with methotrimeprazine (levomepromazine) (0.5 mg/kg, s.c.) and anaesthetized with sodium pentobarbitone (30 mg/kg, i.v.). The animals were artificially ventilated.

Vascular resistance (Willems & Bogaert, 1975) was measured with a pump placed on a catheter loop and the left femoral artery of the hindleg perfused at constant flow. In order to minimize collateral circulation, the right external iliac artery and the caudal aorta were ligated via a small abdominal incision; the left arteria femoralis profunda was also ligated. Circulation to the paw was occluded by means of a tight ligature. Left femoral, obturator and sciatic nerves were cut. Dogs were heparinized (500 u/kg, i.v.) before starting perfusion. Flow was adjusted to obtain an initial perfusion pressure similar to the systemic pressure. Isoprenaline was injected directly into the catheter. The β-adrenoceptor blocking agent was injected into the cephalic vein of the right foreleg 30 min before the assay. The perfusion pressure, recorded distally to the pump, was taken as an index of vascular resistance.

Contractile force was measured by means of a strain gauge sutured on the left ventricle.

Renin activity

Five mongrel dogs were anaesthetized with sodium pentobarbitone. Isoprenaline hydrochloride (Isuprel) was infused into the left renal artery at a dose of 0.1 μg kg^{-1} min^{-1} for 10 min before and for 30 min after administration of IPS 339 at the rate of 6.7 μg kg^{-1} min^{-1} for a period of 30 minutes. Renal blood flow was measured with an electromagnetic flow meter. Renin activity was determined by radioimmunoassay on venous renal blood (Imbs, Kraetz, Schmidt, Desaulles & Schwartz, 1975). Blood pressure and left ventricular dP/dt were measured with a transducer. The blockade of β_2-adrenoceptors was tested by injection of isoprenaline into the renal artery at a dose of 0.25 μg/kg.

Blood pressure variations in rats

Systolic blood pressure was measured with pressure cuffs on the tails of conscious rats.

1. Blood pressure was determined in normotensive rats, 1, 2 and 3 h after oral intake of IPS 339 with three different doses. For each dose, 10 rats were used.
2. Blood pressure was measured in 18 male and 15 female, 6 week-old Okamoto spontaneously hypertensive rats (SHR) divided at random into 3 groups. Intake of IPS 339 (dissolved in 0.9% w/v NaCl solution, 5 ml/kg) took place 6 days out of 7. Systolic blood pressure was measured 24 h after drug intake. The 10 μmol/kg dose of IPS 339 inhibited 23% of the chronotropic effect of intraperitoneal isoprenaline (0.01 μmol/kg) while the 170 μmol/kg dose inhibited 79%.

Local anaesthetic and antiarrythmic activity

Surface anaesthesia technique. To determine local anaesthetic activity we used the technique of Bartsch & Knopf (1970) for measurement of surface anaesthesia of the rabbit cornea.

Antiarrythmic activity. For *in vitro* studies we used a modification of the technique of Dawes (1946). The guinea-pig atria was electrically stimulated at increasing frequencies and we measured the rhythm at which escape appeared.

The aconitine-induced arrythmias technique of Vargaftig & Coignet (1969) was used for *in vivo* studies.

Results

IPS 339 *in vitro* was 155 times more active on the trachea than on the atria of the guinea-pig; pA_2 for

Table 1 β-adrenoceptor blocking effect of IPS 339, practolol and butoxamine *in vitro* and *in vivo*

β-blocking agent		*Isolated atria*		*Isolated trachea*	*Resistance to lung inflation*	*Cardiac rhythm*	*Vascular resistance*	*Contractile force*
		Inotropic effect	*Chronotropic effect*					
IPS 339	$pA_2 \pm$ s.d.	7.03 ± 0.16 (15)	7.04 ± 0.24 (10)	9.23 ± 0.25 (25)	7.45 ± 0.14 (6)	6.03 ± 0.08 (7)	7.48 ± 0.21 (7)	6.12 ± 0.17 (7)
	Slope ± s.d.	−1.49 ± 0.19	−1.32 ± 0.25	−0.66 ± 0.09	−1.28 ± 0.35	−1.39 ± 0.18	−1.23 ± 0.25	−1.46 ± 0.38
Practolol	$pA_2 \pm$ s.d.	6.77 ± 0.31 (8)	6.85 ± 0.12 (10)	5.13 ± 0.30 (12)	4.60 ± 0.07 (5)	6.54 ± 0.14 (7)	4.51 ± 0.11 (5)	6.45 ± 0.10 (5)
	Slope ± s.d.	−1.12 ± 0.26	−1.03 ± 0.09	−0.51 ± 0.12	−0.43 ± 0.11	−1.14 ± 0.18	−0.95 ± 0.25	−1.07 ± 0.08
Butoxamine	$pA_2 \pm$ s.d.	4.82 ± 0.08 (12)	5.34 ± 0.07 (10)	6.44 ± 0.37 (8)	5.04 ± 0.06 (7)	5.25 ± 0.08 (5)	5.16 ± 0.09 (6)	—
	Slope ± s.d.	−1.64 ± 0.26	−1.17 ± 0.10	−0.83 ± 0.17	−0.74 ± 0.13	−0.85 ± 0.12	−1.50 ± 0.30	—

Figures in parentheses show number of measurements taken.

inotropic and chronotropic effects were similar. *In vivo*, its β_2-adrenoceptor selectivity was 23 in dog experiments and 26 in guinea-pig experiments. The β-selectivity is expressed as the antilog of the differences between the pA_2. Table 1 shows the various pA_2 studied and also, for reference, our results with practolol (a β_1-adrenoceptor blocking agent) and butoxamine, the first β_2-blocking agent to be described. We have observed that for each one, selectivity is not the same *in vitro* as *in vivo*. Practolol's β_1-adrenoceptor selectivity was 52 *in vitro*, and 89 *in vivo* for the dog. Butoxamine's β_2-adrenoceptor selectivity was 13 *in vitro* and only 2 *in vivo*. Slopes of the regression lines, were, for the most part, not significantly different from one. It should be pointed out that our experiments were limited in number and also were carried out in the absence of drugs which could prevent aspecific uptake.

IPS 339's local anaesthetic effect was 36 times less than that of (±)-propranolol. The antiarrythmic effect of IPS 339 *in vitro* was the same as that of propranolol. IPS 339 was more potent, mole per mole *in vivo*, in preventing aconitine ventricular fibrillation than quinidine or lidocaïne. It was also significantly more active than propranolol.

Given orally (Table 2) to six-week-old spontaneously hypertensive rats (SHR) each day for six weeks, IPS 339, in β_2-adrenoceptor blocking doses (10 μmol/kg) and in doses 17 times higher (170 μmol/kg) did not prevent the occurrence of arterial hypertension, whereas, under these conditions, the β_1 selective and the non-specific β-adrenoceptor antagonists do prevent it (Weiss, Lundgren & Folkow, 1974). Given orally to conscious SHR at the same two doses, IPS 339 induced an increase in blood pressure which lasted for 6 hours. The increase did not seem dose-dependent (Table 3).

Given orally to conscious normotensive rats, IPS 339 produced a dose-dependent increase in blood pressure which was highest after 2 h (Table 4).

IPS 339 partially inhibited the isoprenaline-induced increases, both in renal blood flow (RBF) and in renal venous renin activity (PRA) (Table 5). Nevertheless, IPS 339 did not decrease either the heart rate, or dP/dt (the difference for this parameter is not significant), or systemic blood pressure.

Discussion

IPS 339 is a new potent β_2-adrenoceptor blocking agent. Butoxamine was the first β_2-blocker described. Later H 35/25 was believed to have a predominant action on the peripheral adrenoceptors. According to Bristow, Sherrod & Green (1970) its selectivity may be related to factors other than differences in receptors. Recently, Todd (1976) suggested that

Table 2 Influence of IPS 339 on the occurrence of arterial hypertension in young spontaneously hypertensive rats (SHR)

			Systolic blood pressure (mmHg ± ts/√n)					
			Weeks of experiment					
IPS 339 (μmol/kg)	n *(number of animals)*	*Control*	1	2	3	4	5	6
Solvent	11	116.4 ± 11.2	123.7 ± 6.7	135.9 ± 7,3*	139.1 ± 4.7**	151.3 ± 10.1**	151.8 ± 10.7**	155.9 ± 13.9**
10	11	114.1 ± 7.3	120.0 ± 5.2	129.6 ± 8.7*	137.7 ± 6.2***	145.5 ± 10.6***	145.6 ± 7.8***	157.7 ± 11.7***
170	11	111.4 ± 11.7	121.7 ± 10.5 (*n* = 9)	135.0 ± 7.4* (*n* = 8)	140.8 ± 6.1* (*n* = 6)	149.0 ± 11.1** (*n* = 5)	153.8 ± 15.0* (*n* = 4)	163.3 ± 51.7 (*n* = 3)

Averages statistically different from the control value; matching *t*-test, *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Table 3 Increase in systolic blood pressure of conscious spontaneously hypertensive rats (SHR) after IPS 339 intake

		Systolic blood pressure (mean ± ts/√n)					
		% increase in relation to control (hours after intake)					
IPS 339 μmol/kg	*Control (mmHg)*	1	2	3	4	5	6
10	132.0 ± 5.6 (10)	13.7 ± 10.5 (9)	16.0 ± 16* (6)	18.0 ± 12.3** (6)	13.2 ± 5.6*** (9)	13.4 ± 8.4** (9)	6.4 ± 5.7** (6)
170	143.0 ± 2.9 (10)	14.1 ± 7.8** (9)	21.4 ± 5.6*** (7)	14.3 ± 9.1* (6)	11.5 ± 5.4* (8)	9.6 ± 6.1* (7)	5.8 ± 5.3* (8)

Figures in parentheses show number of measurements taken.

Averages statistically different from those with the solvent; matching *t*-test, *$P < 0.05$; **$P < 0.01$; ***001.

erythro- and threo-alpha methyl propranolol were more active as vascular rather than as cardiac antagonists. Their selectivity seems to be approximately 10 *in vivo*. They are approximately as active on β_2-receptors as propranolol. IPS 339 is more selective and more potent than propranolol.

For IPS 339, and also for butoxamine and practolol, we obtained not only their *in vitro* pA_2 but also their apparent *in vivo* pA_2. We do not think it is strictly permissible to use *in vivo* injected doses to obtain Schild plots for pA_2 determination. However, in our experiments (Belhadj-Mostefa, 1975) and from Table 1, we can observe the strict similarity of the 'apparent *in vitro* pA_2' of IPS 339, not only on two species but also on two different tissues. On the other hand we have no explanation for the difference of *in vitro* and *in vivo* selectivities. Biodisposability could account for this difference, but this is not borne out by our *in vivo* results.

IPS 339 raises blood pressure in conscious, normotensive or SH rats and does not prevent the occurrence of hypertension in young SHR. β-Blockers generally have an opposite effect. Regoli, Regoli & Gysling (1971) have shown that in urethane anaesthetized rats, intravenous injections of oxprenolol, or propranolol increase arterial pressure, while practolol is ineffective. This pressor effect is not entirely due to the elimination of the β-stimulation of exogenous adrenaline on the vascular system.

Our experiments give no clues as to the explanation of the hypertension observed. From experiments on anaesthetized dogs, it seems that even at β_2-blocking doses, IPS 339 depresses the renin secretion normally induced by isoprenaline. This point needs to be verified extensively. Firstly the nature of β-receptors involved in the control of renin secretion are still subject to discussion. Secondly, our results suggest that the decrease in renin activity cannot account for the fall in blood pressure observed with non-selective beta-blockers.

Table 4 Increase in systolic blood pressure of normal conscious rats after IPS 339 intake

		Systolic blood pressure (mean ± ts/√n)		
IPS 339 (μmol/kg	*Control (mmHg)*	*% increase in relation to control (hours after intake)*		
		1	2	3
10	118.0 ± 4.2	9.1 ± 3.6	10.0 ± 5.1	3.6 ± 3.5
30	98.5 ± 5.0	12.0 ± 7.3	14.4 ± 5.2	12.9 ± 7.6
100	92.0 ± 7.6	16.9 ± 8.7	21.9 ± 8.1	19.6 ± 9.3

For each dose 10 rats were used.
Variation of pressure are dose-dependent at 2 and 3 h (regression analysis).

Table 5 Effects of isoprenaline before and after treatment with IPS 339 (5 anaesthetized dogs)

	Control values	*After IPS 339*
Heart rate (per min)	134 ± 12.5	139 ± 12.5
d*P*/d*t* max (mmHg/s)	2333 ± 391	2016 ± 194
Mean BP (mmHg)	125 ± 8.6	120 ± 7.2
% rise in RBF during isoprenaline infusion	14.7 ± 2.3	4.9 ± 0.9
% rise in renal venous PRA	111 ± 31	26 ± 20

PRA = plasma renin activity; RBF = renal blood flow.

References

BARTSCH, W. & KNOPF, K.W. (1970). Eine modifizierte Methode zur Prüfung der Oberflächenanästhesic an der Kaninchen-Cornea. *Arzneim. Forsch.*, **8**, 1140–1143.

BELHADJ-MOSTEFA, H. (1975). Activité comparée des bêta-bloquants *in vitro* et *in vivo*. *Thèse de Doctorat d'état en biologie humaine*. No. 3. Strasbourg.

BRISTOW, M., SHERROD, T.R. & GREEN, R.D. (1970). Analysis of beta receptor drug interactions in isolated rabbit atrium, aorta, stomach and trachea. *J. Pharmac. exp. Ther.*, **171**, 52–61.

DAWES, G.S. (1946). Synthetic substitutes for quinidine. *Br. J. Pharmac. Chemother.*, **1**, 90–111.

HORII, D., KAWADA, T., TAKEDA, K. & IMAI, S. (1974). Comparison of beta-adrenergic blocking activities of propranolol, isopropyl methoxamine, sotalol, practolol, alprenolol, pindolol, oxprenolol and D-32 in the atria and trachea of the guinea-pig. *Arzneim. Forsch.*, **24**, 1275–1277.

IMBS, JL., KRAETZ, J., SCHMIDT, M., DESAULLES, E. & SCHWARTZ, J. (1975). Beta-blocking drugs and renin secretion in the anaesthetized dog. *Clin. Sc. Mol. Med.*, **48**, 105s–107s.

KONZETT, H. & RÖSSLER, R. (1940). Versuchsanordnung zu Untersuchungen an der Bronchialmuskulatur. *Naunyn-Schmiedeberg's Arch. Exp. Path. Pharmak.*, **195**, 71.

LEVY, B. & WILKENFELD, B.E. (1970). Reactions of selective beta-receptor antagonists on the guinea pig trachea. *Eur. J. Pharmac.*, **11**, 67–74.

MANN, A. (1975). Antagonistes des récepteurs adrénergiques. Synthèse et pharmacologie. *Thèse de "Docteur-Ingénieur"*. Strasbourg.

REGOLI, D., REGOLI, U. & GYSLING, E. (1971). Pressor effect of beta-blocking agents in rats. *Can. J. Physiol. Pharmac.*, **50**, 207–214.

TODD, M.H. (1976). The activity of alpha-methyl substituted beta-adrenoceptor antagonists in the rat. *Pharmacologist*, **18**, 138.

VARGAFTIG, B. & COIGNET, J.L. (1969). Critical evaluation of three methods for the study of adrenergic beta-blocking anti-arrythmic agents. *Eur. J. Pharmac.*, **6**, 49–55.

WEISS, L., LUNDGREN, Y. & FOLKOW, B. (1974). Effects of prolonged treatment with adrenergic beta-receptor antagonists on blood pressure, cardiovascular design and reactivity in spontaneously hypertensive rats (SHR). *Acta physiol. scand.*, **91**, 447–457.

WILLEMS, J.L. & BOGAERT, M.G. (1975). Dopamine induced neurogenic vasodilatation in isolated perfused muscle preparation of the dog. *Naunyn-Schmiedeberg's Arch. Pharmac.*, **286**, 413–428.

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