J. Pharm. Pharmacol. 1985, 37: 410–414
Received October 16, 1984

Peripheral cardiovascular α- and β-adrenergic effects of some hypotensive and bradycardic arylalkyl imidazole derivatives in the rat†

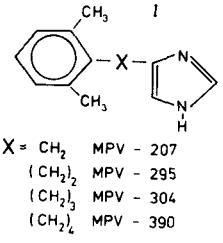
J.-M. SAVOLA*, J. PUURUNEN, H. RUSKOAHO AND N. T. KÄRKI

Department of Pharmacology, University of Oulu, Kajaanintie 52 D, SF-90220 Oulu, Finland

In pithed rats, a series of four alkyl bridge analogues of 4(5)-substituted arylalkyl imidazole induced α-adrenoceptor-mediated vasoconstriction and inhibition of electrically stimulated tachycardia. These effects were induced in the order of potency clonidine = MPV 207 > MPV 295 > MPV 304 > MPV 390, correlating with the length of the alkyl bridge between the phenyl and imidazole moieties. The peripheral postsynaptic actions of MPV 207 and MPV 304 were attenuated by prazosin (0·1 mg kg^{-1} i.v.) and yohimbine (1 mg kg^{-1} i.v.). The pressor responses induced by MPV 295 were antagonized only by yohimbine (0·3 and 1 mg kg^{-1} i.v.). The peripheral sympathoinhibitory action of these compounds was antagonized by yohimbine (1 mg kg^{-1} i.v.). In spontaneously beating rat atria, the MPV compounds showed neither agonistic nor antagonistic activity at cardiac postsynaptic α- and β-adrenoceptors. The results indicate that the hypotensive and bradycardic MPV compounds are agonists at peripheral cardiovascular α-adrenoceptors. The extension of the alkyl bridge between the phenyl and imidazole moieties reduces their activity at α-adrenoceptors. Finally, MPV 295 seems to be a selective agonist of peripheral α_2-adrenoceptors in the cardiovascular system of the pithed rat.

A number of compounds with the general structure of 4(5)-substituted arylalkyl imidazoles are hypotensive and reduce the heart rate in rats and cats, one of the most effective being 4(5)–(2,6-dimethylbenzyl)-imidazole (MPV 207) (Puurunen et al 1982, 1983). The pattern of cardiovascular action resembles that of clonidine (Schmitt 1977) and there is evidence that MPV 207 and its analogue MPV 295 (I) act in the central nervous system to reduce blood pressure and heart rate by stimulating α_2-adrenoceptors (Kaipiainen et al 1982; Puurunen et al 1983; Ruskoaho et al 1983) like clonidine (Hamilton et al 1980; Timmermans et al 1981). MPV 207 and MPV 295 have therefore been introduced to clinical trials.

The mechanisms of the cardiovascular action of MPV 207 were examined here by studying its effects on peripheral α-adrenoceptors in the pithed rat. The effect of the length of the alkyl bridge between the phenyl and imidazole moieties for the α-adrenergic activities of the compound was then investigated using three alkyl bridge analogues (I). Furthermore, isolated spontaneously beating atrial preparations from the rat were used to study in-vitro whether these compounds exert α-adrenoceptor mediated positive chronotropic activity (Flavahan & McGrath 1981, 1982) and, finally, to exclude β-adrenoceptor blocking potency.



X = CH_2 MPV - 207
$(CH_2)_2$ MPV - 295
$(CH_2)_3$ MPV - 304
$(CH_2)_4$ MPV - 390

I. Structural formulae of the MPV compounds.

METHODS

Male Wistar rats (250–350 g) were pithed under ether anaesthesia after atropine sulphate (1 mg kg^{-1} s.c.). The uncovered tip of the pithing rod (φ 2·1 mm) was placed at the level C7-T1, the origin of the spinal sympathetic cardioaccelerator nerve roots (Gillespie et al 1970). An indifferent electrode was inserted under the back skin. Artificial respiration was provided with a Parker ventilation pump (52 strokes min^{-1}, 1 ml/100 g) using 95% oxygen and 5% carbon dioxide. The arterial blood gas analysis (Radiometer Copenhagen device) performed after

† Parts of this work was presented as preliminary results to Joint Meeting of the British and Scandinavian Pharmacological Societies (Savola et al 1982) and to 5th International Catecholamine Symposium, Göteborg, June 12–16, 1983.

* Correspondence.

2 h of concurrent work with a test compound revealed the following values: pH 7·2, pO_2 19·3 kPa, pCO_2 8·8 kPa, HCO_3 22·4 mM (n = 6). The left femoral vein was cannulated for drug administration, and the rats received heparin 1000 IU kg^{-1} i.v. Heart rate (HR) and mean arterial pressure (MAP) were recorded continuously from the femoral artery on a Grass Polygraph (model 7 D) via a Micron pressure transducer (MP-15). The rectal temperature of the animals was maintained at 37 °C. At least 20 min was allowed to elapse after preparation for HR, MAP and body temperature to become stabilized.

5 min after the i.v. injection of the substances (0·5 ml kg^{-1} within 75 s) the HR was elicited with a Palmer electronic square wave stimulator via a pithing rod (20 impulses, 0·3 ms, 0·4 Hz, 50 V) at 20-min intervals. The doses were given as single doses in increasing order, each rat receiving one test compound only.

The doses inducing an increase of 50 mmHg in MAP (PD50) and a 50% inhibition of electrically stimulated tachycardia (*ID50*) were determinated graphically from the log dose-response curves.

Spontaneously beating isolated rat atria

Female Wistar rats (200–250 g) were killed by a blow on the neck and exsanguinated. The chest was opened, the heart rapidly removed and placed in Krebs-Henseleit solution (mM: NaCl 118·0, KCl 4·7, $NaHCO_3$ 22·1, $MgSO_4$ 1·26, KH_2PO_4 1·20, $CaCl_2$ 2·5 and glucose 11·1) oxygenated via a sintered disc. After dissection, the atrial preparation, the appendages being tied at their tips with a cotton thread, was mounted in a 10 ml organ bath (37 °C) containing the continuously gassed (95% O_2–5% CO_2) Krebs-Henseleit solution. The spontaneously beating atria were suspended under a resting tension of 0·4 g, their beating frequency being recorded via the Grass Polygraph by means of a Grass FT.03 isometric transducer. The atria were allowed to stabilize for 60 min (with changes in bath fluid every 20 min) before commencement of the experiment.

Cumulative dose responses were obtained for isoprenaline (3×10^{-10}–3×10^{-8} M) at 0·5 $\log_{10}$ intervals and for clonidine and the MPV compounds (10^{-9}–10^{-5} M) at $\log_{10}$ intervals. In the absence of any response, 3 min was allowed to elapse before adding the next dose. The positive chronotropic effect of isoprenaline was measured in terms of the increase from the basal beating frequency and calculated as a percentage of the maximum response. The changes in the rate of beating induced by the test compound were measured in a similar way and expressed as percentages of the maximum of the preceding control concentration-response curve for isoprenaline. The affinities (pD_2) were determinated from the cumulative dose response curves as described by van Rossum (1963).

The antagonistic action of clonidine and the MPV compounds on the atrial β-adrenoceptors were studied by adding a test compound (10^{-5} M) to the bath for 10 min, after which a new cumulative dose–response curve for isoprenaline was recorded. The statistical analysis was performed using a paired *t*-test.

Drugs

The MPV compounds as HCl (Farmos Research Center) and clonidine HCl (Star, Tampere, Finland) were dissolved in saline. Prazosin HCl (Orion Pharmaceutical Co, Espoo, Finland) and yohimbine HCl (Sigma) were dissolved in distilled water. Isoprenaline $H_2SO_4.2H_2O$ (Farmos) was dissolved in 20 μM ethylenediaminetetraacetic acid and stored on ice. Dilutions for the atrial preparation were made with the Krebs-Henseleit solution.

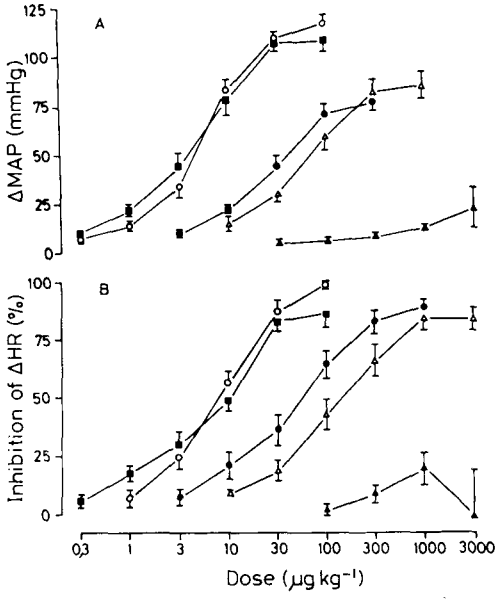


FIG. 1. Dose–response curves for clonidine and the MPV compounds, drugs given as single doses, with respect to (A) increase in mean arterial pressure (ΔMAP) and (B) electrically stimulated tachycardia: percentage inhibition of heart rate in pithed rats. Clonidine (■); MPV 207 (○); MPV 295 (●); MPV 304 (△); MPV 390 (▲) (means ± s.e.m., n = 6–10).

RESULTS

Pithed rats

Intravenous injection of the MPV compounds induced vasopressor responses (Fig. 1A) and inhibition of electrically stimulated tachycardia (Fig. 1B) in a dose-dependent manner. The initial levels (means ± s.e.m.) for MAP and HR were 59·0 ± 0·9 mmHg and 369 ± 9 beats min^{-1} (n = 130). The tachycardia elicited by the train of 20 electrical impulses was 106 ± 2 beats min^{-1} (means ± s.e.m., n = 130), the response being reproducible for the duration of the experiment.

MPV 207 was as potent as clonidine and replacement of the methano bridge of MPV 207 with ethano and propano groups as in MPV 295 and MPV 304, respectively, reduced the vasopressor and sympathoinhibitory activities (Table 1). MPV 390 with a butano junction was practically inactive.

The α_1- and α_2-adrenoceptor antagonists prazosin (0·1 mg kg^{-1} i.v., −10 min) and yohimbine (1 mg kg^{-1} i.v., −10 min), respectively, attenuated the pressor responses of MPV 207 and MPV 304. The vasopressor response of MPV 295, in turn, was antagonized by yohimbine at doses 0·3 mg kg^{-1} (data not presented) and 1 mg kg^{-1} i.v., whereas prazosin (0·1 mg kg^{-1}) was without effect. (Fig. 2).

The inhibitory effect of clonidine and the MPV

Table 1. Vasopressor and sympathoinhibitory effects of clonidine and the MPV compounds in pithed rats (n = 6–10).

Compound	PD50[a]	ID50[b]
Clonidine	3·9	7·3
MPV 207	4·5	7·8
MPV 295	42	53
MPV 304	74	143

[a] Dose (μg kg^{-1}) which increases mean arterial pressure in pithed rats by 50 mmHg.
[b] Dose (μg kg^{-1}) which inhibits electrically stimulated tachycardia in pithed rats by 50%.

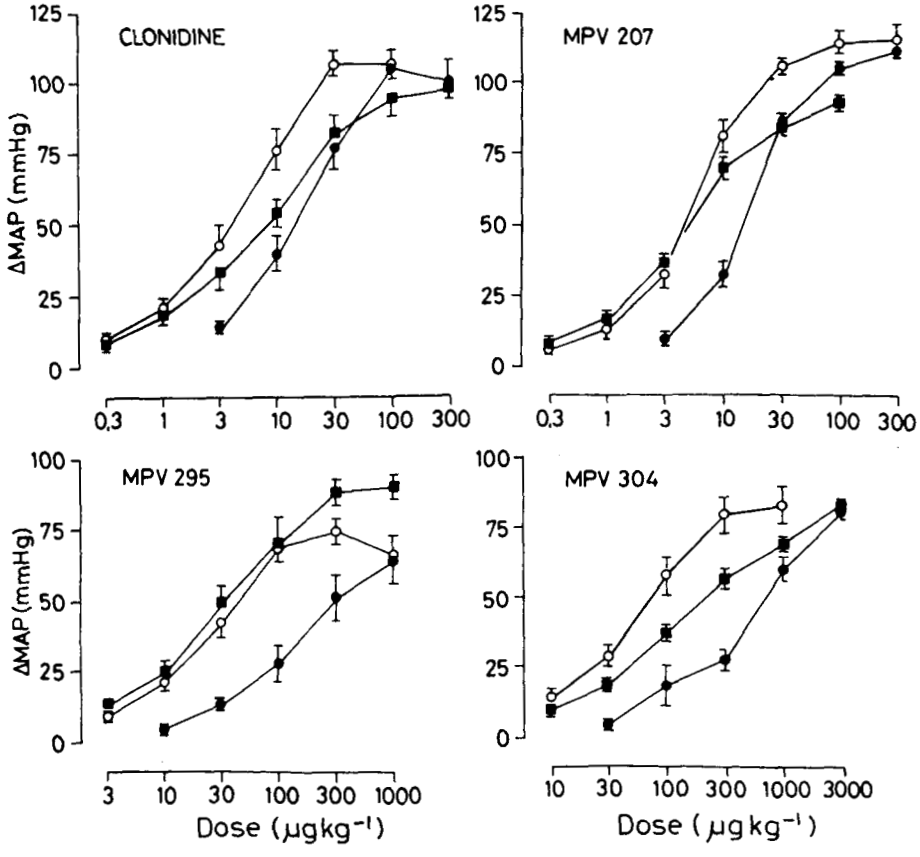


FIG. 2. Influence of prazosin (0·1 mg kg^{-1} i.v., 10 min before (■)) and yohimbine (1 mg kg^{-1} i.v., 10 min before (●)) on vasopressor activities of clonidine, MPV 207, MPV 295 and MPV 304 in pithed rats; dose response curve without antagonist (○). (Means ± s.e.m., n = 6–10.)

compounds on electrically stimulated tachycardia was effectively antagonized by yohimbine (1 mg kg^{-1} i.v.), whereas prazosin (0·1 mg kg^{-1} i.v.) had no effect. 13–16-fold displacements of the dose response curves to the right were recorded.

Spontaneously beating isolated rat atria

The basal beating frequency (means ± s.e.m.) of the atria (247 ± 7 beats min^{-1}, n = 20) was accelerated by isoprenaline, the pD_2 being 8·52 ± 0·17 (n = 20). The chronotropic response to isoprenaline was not affected either by clonidine or by the MPV compounds at 10^{-5} M concentrations ($P > 0·05$, paired *t*-test).

The substances by themselves had no intrinsic activity on the atria in the dose range 10^{-9}–10^{-5} M, although the highest concentration of clonidine tested (10^{-5} M) had a slight negative chronotropic effect, the beating frequency falling from an initial value of 269 ± 6 to 235 ± 2 beats min^{-1} (n = 4; $P < 0·05$).

DISCUSSION

We have previously shown that MPV 207 and some of its alkyl bridge analogues are hypotensive and bradycardic in anaesthetized rats (see Introduction). The results of the present study show that these MPV compounds are agonists at α-adrenoceptors, since α-blocking drugs prazosin and yohimbine antagonized the vasopressor responses and inhibition of electrically stimulated tachycardia observed in pithed rats.

Parallelling the hypotensive and bradycardic activities in intact, urethane-anaesthetized rats (Puurunen et al 1983), the peripheral hypertensive and sympathoinhibitory activities of these 4(5)-substituted arylalkyl imidazoles was reduced in proportion to the extension of the alkyl bridge in the ring junction, MPV 390 with a butano bridge between the phenyl and imidazole moieties being practically without effect. Previously Ruffolo et al (1980) have observed a similar decrease in efficacy and affinity due to the extension of the alkyl bridge in tolazoline-like α-adrenergic imidazolines. The loss of effect of MPV 295 and MPV 304 compared to MPV 207 seemed to be due to loss of stiffness of the molecules as the two conformation-stabilizing nuclei are brought further apart (Karjalainen 1981).

Being attenuated by α-adrenoceptor antagonists, the vasopressor action of the MPV compounds must be, at least to an appropriate extent, due to stimulation of the peripheral postsynaptic α-adrenoceptors. The results obtained here also provide evidence for more than one type of postjunctional α-adrenoceptor, as been previously reviewed by e.g. Timmermans & van Zwieten (1981) and McGrath (1982).

4(5)-(2,6-Dimethylbenzyl)imidazole (MPV 207) brought about vasoconstriction of similar magnitude and potency to that achieved with clonidine. The rises in MAP after i.v. injection of the drugs were attenuated in the same manner by prazosin, an α_1-adrenoceptor antagonist (Cambridge et al 1977), and yohimbine, a preferential antagonist at α_2-adrenoceptors (Starke et al 1975). The vasopressor response induced by MPV 304, the propano bridge analogue of MPV 207, was also antagonized by the both α-adrenoceptor blocking drugs, to an even greater extent than with the other substances. The non-linear displacement of the dose-response curve of MPV 207 and MPV 304 resembles the results obtained previously concerning clonidine (Timmermans & van Zwieten 1980), guanfacine (Timmermans et al 1979) and UK-14,304 (Cavero et al 1982) and is attributed to the stimulation of α_2-adrenoceptors at low concentrations of the drugs and of both α_1- and α_2-adrenoceptors at high concentrations.

The peripheral pressor effect of MPV 295, which has an ethano bridge between the phenyl and imidazole moieties, was antagonized by yohimbine but not by prazosin. When the proposed α_2-agonism was subsequently studied using 0·3 mg kg^{-1} yohimbine, a dose which will antagonize the hypertensive action of azepexole but not attenuate the pressor response induced by phenylephrine, the result implied that MPV 295 is selective for peripheral α_2-adrenoceptors in pithed rats, confirming the results reported previously (Ruskoaho et al 1983). Thus, MPV 295 might be a suitable pharmacological tool to study the α_2-adrenoceptor mediated effects.

The rat heart has been reported to contain postsynaptic α_1-adrenoceptors, the stimulation of which leads to a positive chronotropic effect (Flavahan & McGrath 1981, 1982) although contrary reports have also been presented (Kunos 1977; Wagner & Brodde 1978; Bennett & Kemp 1978). The present results are compatible with those of Stene-Larsen (1980), since the imidazoles studied, although active at postsynatic vascular α_1-adrenoceptors, had no consistent positive chronotropic effect upon spontaneously beating rat atria in the dose-range 10^{-9}–10^{-5} M. Neither the compounds under the study had any β-adrenoceptor blocking

activity at a concentration 10^{-5} M, which was considered to be compatible with the concentrations underlying the pharmacological actions in-vivo, at least. Clonidine showed some slight negative chronotropic action in the atria at its highest concentration (10^{-5} M), but the mechanism of this effect was not investigated further.

In conclusion, extension of the alkyl bridge between the phenyl and imidazole moieties of the MPV compounds reduced the activity upon peripheral α-adrenoceptors, a finding which parallels their hypotensive and bradycardic activities. MPV 295 was a selective compound towards α_2-adrenoceptors. The results obtained in the pithed rat provide further evidence for heterogeneity among the postsynaptic α-adrenoceptors. The possibility of the bradycardic and sympathoinhibitory action of the compounds being of antagonism at β-adrenoceptors was ruled out using spontaneously beating rat atria.

Acknowledgement

The excellent assistance of Ms Tuula Räisänen is gratefully acknowledged.

REFERENCES

Bennett, T., Kemp, P. A. (1978) Naunyn-Schmiedeberg's Arch. Pharmacol. 301: 217–222

Cambridge, D., Davey, M. J., Massingham, R. (1977) Br. J. Pharmacol. 59: 514P–515P

Cavero, I., Lefèvre-Borg, F., Roach, A. G., Gomeni, R., Scatton, B. (1982) J. Pharmacol. Exp. Ther. 223: 241–250

Flavahan, N. A., McGrath, J. C. (1981) Br. J. Pharmacol. 73: 586–588

Flavahan, N. A., McGrath, J. C. (1982) Ibid. 77: 319–328

Gillespie, J. S., MacLaren, A., Pollock, D. (1970) Ibid. 40: 257–267

Hamilton, T. C., Hunt, A. A. E., Poyser, R. H. (1980) J. Pharm. Pharmacol. 32: 788–789

Kaipiainen, S., Puurunen, J., Ruskoaho, H., Kärki, N. T. (1982) Br. J. Pharmacol. 77: 370P

Karjalainen, A. (1981) Acta Univ. Oul. A 125 Chem. 15

Kunos, G. (1977) Br. J. Pharmacol. 59: 177–189

McGrath, J. C. (1982) Biochem, Pharmacol. 31: 467–484

Puurunen, J., Kärki, N., Ruskoaho, H., Karjalainen, A., Kurkela, K. (1982) Naunyn-Schmiedeberg's Arch. Pharmacol. 319: R49

Puurunen, J., Ruskoaho, H., Kärki, N. T., Karjalainen, A., Kurkela, K. (1983) Arch. Int. Pharmacodyn. 261: 102–109

Ruffolo, R. R., Jr., Yaden, E. L., Waddell, J. E., Dillard, R. D. (1980) J. Pharmacol. Exp. Ther. 214: 535–540

Ruskoaho, H., Savola, J.-M., Kaipiainen, S., Puurunen, J., Kärki, N. T. (1983) Naunyn-Schmiedeberg's Arch. Pharmacol. 322: 279–285

Savola, J.-M., Puurunen, J., Ruskoaho, H., Kärki, N. T. (1982) Br. J. Pharmacol. 77: 318P

Schmitt, H. (1977) in: Gross, F. (ed.) Antihypertensive agents, Handbuch der experimentellen Pharmacologie. Springer Verlag, Berlin Heidelberg New York, vol. 39, pp 299–396

Starke, K., Borowski, E., Endo, T. (1975) Eur. J. Pharmacol. 34: 385–388

Stene-Larsen, G. (1980) Gen. Pharmacol. 11: 575–581

Timmermans, P. B. M. W. M., Kwa, H. Y., van Zwieten, P. A. (1979) Naunyn-Schmiedeberg's Arch. Pharmacol. 310: 189–193

Timmermans, P. B. M. W. M., McSchoop, A., Kwa, H. Y., van Zwieten, P. A. (1981) Eur. J. Pharmacol. 70: 7–15

Timmermans, P. B. M. W. M., van Zwieten, P. A. (1980) Ibid. 63: 199–202

Timmermans, P. B. M. W. M., van Zwieten, P. A. (1981) J. Auton. Pharmacol. 1: 171–183

van Rossum, J. M. (1963) Arch. Int. Pharmacodyn. 143: 299–330

Wagner, J., Brodde, O.-E. (1978) Naunyn-Schmiedeberg's Arch. Pharmacol. 302: 239–254