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Cardiovascular effects of two new calcium antagonists, PY 108-068 and PN 200-110, in conscious spontaneously hypertensive rats

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1 The acute cardiovascular effects of PY 108-068 and PN 200-110 were studied by means of a computerized analysis of the intra-aortic blood pressure (BP) recorded continuously for 26 h in conscious unrestrained spontaneously hypertensive rats. Both compounds were studied at three doses (50, 100 and 200 $\mu g\,kg^{-1}$) and each dose was administered intravenously 5 times (09 h 00 min, 14 h 00 min, 19 h 00 min, 24 h 00 min and 09 h 00 min). Baroreflex sensitivity was measured 1 h following the last injection.

2 The two compounds were found to induce rapid (3 min) and dose-dependent falls in BP. After the first administration, these decreases reached −20% and −35% for systolic BP (SBP) and diastolic BP (DBP) respectively with PY 108-068 (200 $\mu g\,kg^{-1}$) and −25% and −45% for SBP and DBP respectively with PN 200-110 (200 $\mu g\,kg^{-1}$).

3 The duration of the reduction in BP increased with the dose and was much longer for PN 200-110 (180 min for SBP) than for PY 108-068 (20 min for SBP).

4 A tachycardia was associated with the decrease in BP which did not differ at 200 $\mu g\,kg^{-1}$ between PY 108-068 (+ 108 beats min^{-1}) and PN 200-110 (+ 103 beats min^{-1}). Baroreflex sensitivity was not significantly increased by either drug.

5 The 5 repeated injections of PY 108-068 and PN 200-110 evoked similar responses.

6 In conclusion, both compounds exhibited marked hypotensive properties. PN 200-110 appeared to be more suitable for further development since its effects were found to be greater and much longer lasting than those of PY 108-068.

Introduction

Calcium entry blockers, by their direct action on vascular smooth cells are potent vasodilators (Cauvin *et al.*, 1983) which are increasingly used in the treatment of hypertension (Spivack *et al.*, 1983). The drug-induced fall in blood pressure (BP) is normally associated with a baroreflex-mediated tachycardia despite the negative chronotropic intrinsic effect frequently exhibited by calcium entry blockers. However, this tachycardia, which partly counteracts the hypotensive action, has been reported to decrease during prolonged administrations (Spivack *et al.*, 1983).

In the present work we studied the kinetics of the cardiovascular effects of PY 108-068 (diethyl-4-(2,1,3-benzoxadiazol-4-yl)-1,4-dihydro-2,6-dimethyl-pyridine-3,5-dicarboxylate) and PN 200-110 (isopropyl-4-(2,1,3-benzoxadiazol-4-yl)-1,4-dihydro-5-methoxy carbonyl-2,6-dimethyl-3-pyridine carboxylate), two new dihydropyridine derivatives with potent and selective calcium antagonist properties (Hof *et al.*, 1982; 1984a,b). Since PY 108-068 or PN 200-110 which, *in vitro*, exhibit negative chronotropic effects (Hof & Scholtysik, 1983; Hof *et al.*, 1984b), have been shown to decrease heart rate (HR) in anaesthetized cats (Hof *et al.*, 1982; 1984a) but to increase it in conscious animals (Hof & Scholtysik, 1983; Nievelstein *et al.*, 1985), our study was performed in freely moving rats. In addition, the method used allowed, as previously described (Cerutti *et al.*, 1985), measurement of diastolic blood pressure (DBP), a parameter which is of special importance for the assessment of the effect of vasodilator drugs.

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Adult spontaneously hypertensive rats (SHR) from the Japanese strain, known to be highly sensitive to calcium antagonists (Pegram *et al.*, 1984; Nagura *et al.*, 1986), were used. In order to assess the influence of repeated administration, each animal received several injections of PY 108-068 or PN 200-110 during which their BP and HR were continuously monitored. At the end of the study the effects of both drugs on the cardiac baroreflex sensitivity were measured.

Methods

Animals

Thirteen-week-old male SHR were purchased from Iffa Credo (Les Oncins, France) and kept for two weeks of habituation in our animal house (temperature 21 ± 1°C; humidity: 60 ± 10%; lighting: 08 h 00 min–20 h 00 min). Standard laboratory animal food containing less than 0.2% of sodium (UAR Entretien, France) and tap water were provided *ad libitum*.

Protocol

Fifteen-week-old SHR were randomly divided into six treated groups ($n = 5$ each) and one control group ($n = 6$). Three doses (50, 100 and 200 $\mu g\,kg^{-1}$) of PY 108-068 and PN 200-110, chosen on the basis of previous experiments, were studied, each in a single group. The control group received solvent.

Chronically instrumented rats were placed in observation cages for a 12 h habituation period. Afterwards, BP and HR were recorded continuously for 26 consecutive h (08 h 00 min–10 h 00 min) in conscious unrestrained rats. During this period PY 108-068, PN 200-110 or solvent were injected at 09 h 00 min, 14 h 00 min, 19 h 00min, 24 h 00 min and 09 h 00 min in each rat. Finally, 1 h after the last injection, the baroreflex sensitivity was assessed after several i.v. injections of phenylephrine (5 $\mu g\,kg^{-1}$) and nitroglycerin (100 $\mu g\,kg^{-1}$).

Blood pressure and heart rate recordings

These were obtained by our previously described technique (Cerutti *et al.*, 1985). Briefly, in halothane-anaesthetized rats, a floating polyethylene catheter (PE20; 0.4 mm internal diameter) was inserted into the lower abdominal aorta for BP recording and another one into the jugular vein for i.v. injections. During the recording sessions the aortic catheter was connected to a BP transducer (Statham P 23 ID) by means of a rotating swivel (Ets R.B., Lyon, France) and a three-way stop-cock which allowed a continuous perfusion of the catheter with heparinized (25 u ml^{-1}) isotonic glucose solution (flow rate: 0.5 ml h^{-1}). The BP signal was digitized and processed by a mini computer (PDP 11–23, Digital Equipment Co.) which calculated on-line the following parameters: systolic BP (SBP, mmHg), DBP (mmHg) and HR (beats min^{-1}). These data were stored on hard disks for off-line processing including graphical treatments and statistical analysis.

Data processing

The base-line values, defined as the mean of the data obtained during the first hour (08 h 00 min–09 h 00 min) of recording, exhibited (see Table 1) slight but significant differences between treated and control groups. Therefore, for comparisons to be made, the BP and HR of treated and control rats were expressed as percentage of the value observed, as a mean, during the hour (08 h 00 min–09 h 00 min) which preceded the first drug administration. These data, averaged every 3 min, were used to construct the chronograms which allowed the determination of the kinetics of the effects of PY 108-068 and PN 200-110. In addition, dose-response curves were obtained by use of the absolute differences between the control period values and those corresponding to the maximum effect of the first drug administration. The effects of the subsequent injections were similarly calculated in order to assess the influence of repeated administrations.

Baroreflex sensitivity

The measurement of the baroreflex sensitivity was by the method of Smyth *et al.* (1969) with minor modifications. Changes in SBP and heart period (60 000/HR ms) observed after several injections of phenylephrine and nitroglycerin were recorded beat by beat. For each injection, the relationship (ms $mmHg^{-1}$) between SBP and heart period changes was computed with 16 different phase-shifts (from 0 to 15 beats). The closest relationship obtained was used as an index of the baroreflex sensitivity. When the significance of the correlation coefficient did not reach the $P < 0.001$ level, the experiment was discarded.

Statistical analysis

Results are expressed as mean ± s.e.mean. Statistical comparisons used the non-parametric Mann and Whitney test (Conover, 1971).

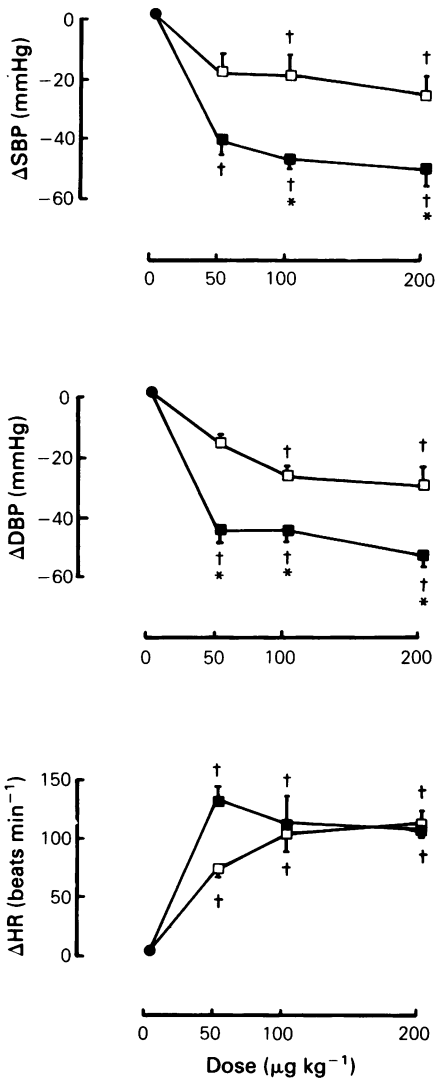
Drugs

Stock solutions of PY 108-068 or PN 200-110 (Sandoz) were made in a mixture of ethanol and polyethylene glycol 400 (0.5 mg ml^{-1} of active substance). These solutions were diluted with saline in order to make injections in the same volume (200 μl).

Table 1 Baseline values for systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR)

Dose ($\mu g\,kg^{-1}$)		*SBP* (mmHg)	*DBP* (mmHg)	*HR* (beats min^{-1})
Controls	(6)	169 ± 1.7	104 ± 3.9	318 ± 11.3
PY 108-068 50	(5)	172 ± 7.5	107 ± 9.1	328 ± 7.1
PY 108-068 100	(5)	179 ± 4.8	119 ± 4.7	319 ± 6.3
PY 108-068 200	(5)	163 ± 1.8*	97 ± 3.1	330 ± 16.1
PN 200-110 50	(5)	174 ± 6.1	112 ± 7.1	322 ± 11.2
PN 200-110 100	(5)	174 ± 5.3	108 ± 8.4	327 ± 18.8
PN 200-110 200	(5)	171 ± 4.6	115 ± 4.2	314 ± 7.2

Values are given as the mean (± s.e.mean) value observed during the first hour of recording in control and PY 108-068 or PN 200-110 treated groups of rats. The number of animals is in parentheses. * = $P < 0.05$ versus controls.



Results

Dose-response curves for PY 108-068 andPN 200-110

Table 1 summarizes the base-line values of SBP, DBP and HR obtained, as a mean, during 1 h (08 h 00 min–09 h 00 min) before treatment in the different groups of SHR used. It indicates that, for an unexplained reason, the SHR which received PY 108-068 at a dose of 200 $\mu g\,kg^{-1}$ differed slightly but significantly from the others. Consequently, the dose-responses curves used the absolute differences observed between the baseline and the peak effect values. As shown in Figure 1, the first administration of both drugs induced falls in BP which were more marked for DBP than for SBP and were associated with an increased HR. PY 108-068 peak-effects were dose-related and were less than those of PN 200-110. In the case of PN 200-110, the dose-response relationship did not appear clearly since the lowest dose used (50 $\mu g\,kg^{-1}$) almost induced the maximum effect. Despite a greater hypotensive action, the tachycardia induced by PN 200-110 was similar to that observed with PY 108-068. In addition, when the dose of PN 200-110 was increased, the effect on BP tended to increase and that on HR to decrease.

Influence of repeated injections on the responses to PY 108-068 and PN 200-110

The injections of each dose of PY 108-068 or of PN 200-110, which were administered, during 24 h, in

Figure 1 Mean dose-response curves for systolic blood pressure (SBP), diastolic BP (DBP) and heart rate (HR) observed in spontaneously hypertensive rats after the first injections of PY 108-068 (□) and PN 200-110 (■); vertical lines show s.e.mean. The response (Δ) is the difference between the baseline value and that observed at the peak of the drug effect. $n = 5$ in each group. † $P < 0.05$ versus baseline value. * $P < 0.05$ PN 200-110 versus PY 108-068.

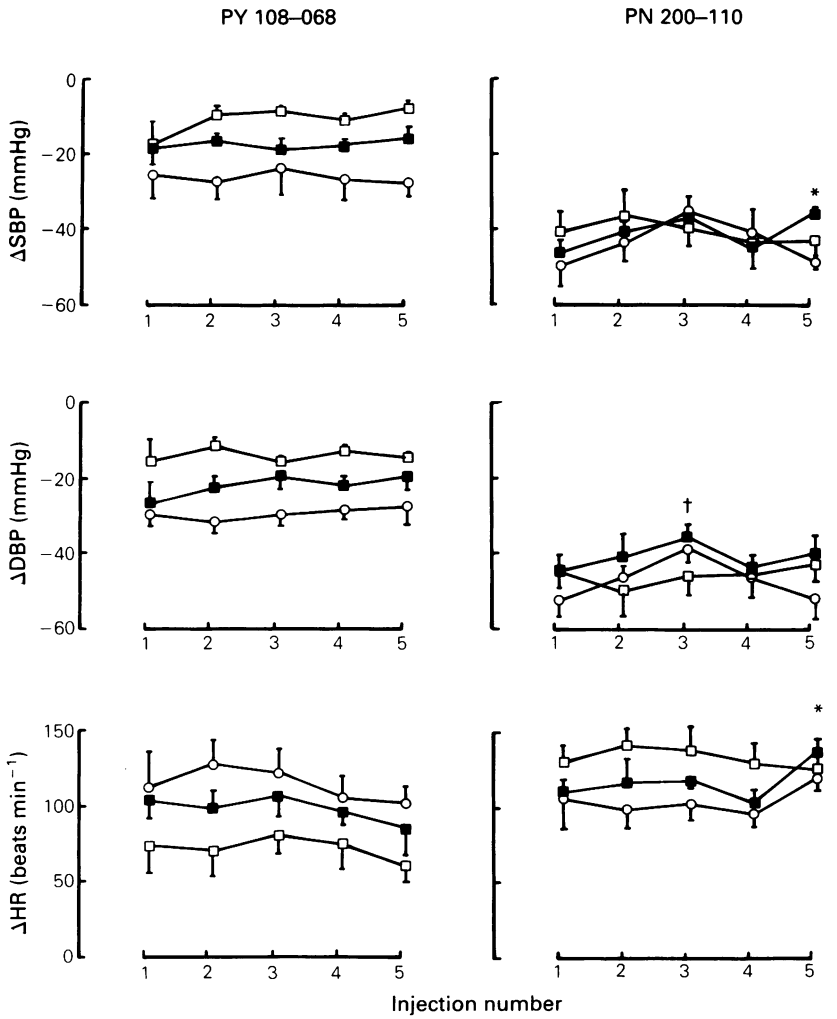


Figure 2 Responses (mean) to 5 successive injections of PY 108-068 (left panels) and PN 200-110 (right panels) on systolic blood pressure (SBP), diastolic BP (DBP) and heart rate (HR) of spontaneously hypertensive rats. The response (Δ) is the difference between base line value and that observed at the peak of the effect. Injections number 1 to 5 were made at 09 h 00 min, 14 h 00 min, 19 h 00 min, 24 h 00 min and 09 h 00 min respectively. For each compound the doses used were 50 (□), 100 (■) and 200 $\mu g\,kg^{-1}$ (○). $*P < 0.05$ versus the first injection.

resting (day-time, injections number 1, 2, 3, 5) and in active (night-time, injection number 4) rats, induced reproducible maximum effects (see Figure 2).

Time course of the effects of PY 108-068 and PN 200-110

As shown by Figure 3, the highest dose of PY 108-068 used induced sharp, reproducible and short-lasting decreases in SBP and DBP associated with increases in HR. The short duration of these effects could not be precisely determined using such a chronogram made with values averaged each 3 min. Therefore, chronograms of the data averaged over each min were drawn which allowed the demonstration (see Figure 4) that the effects of the first injection of PY 108-068 (200 $\mu g\,kg^{-1}$, i.v.) reached their maximum within 3 min

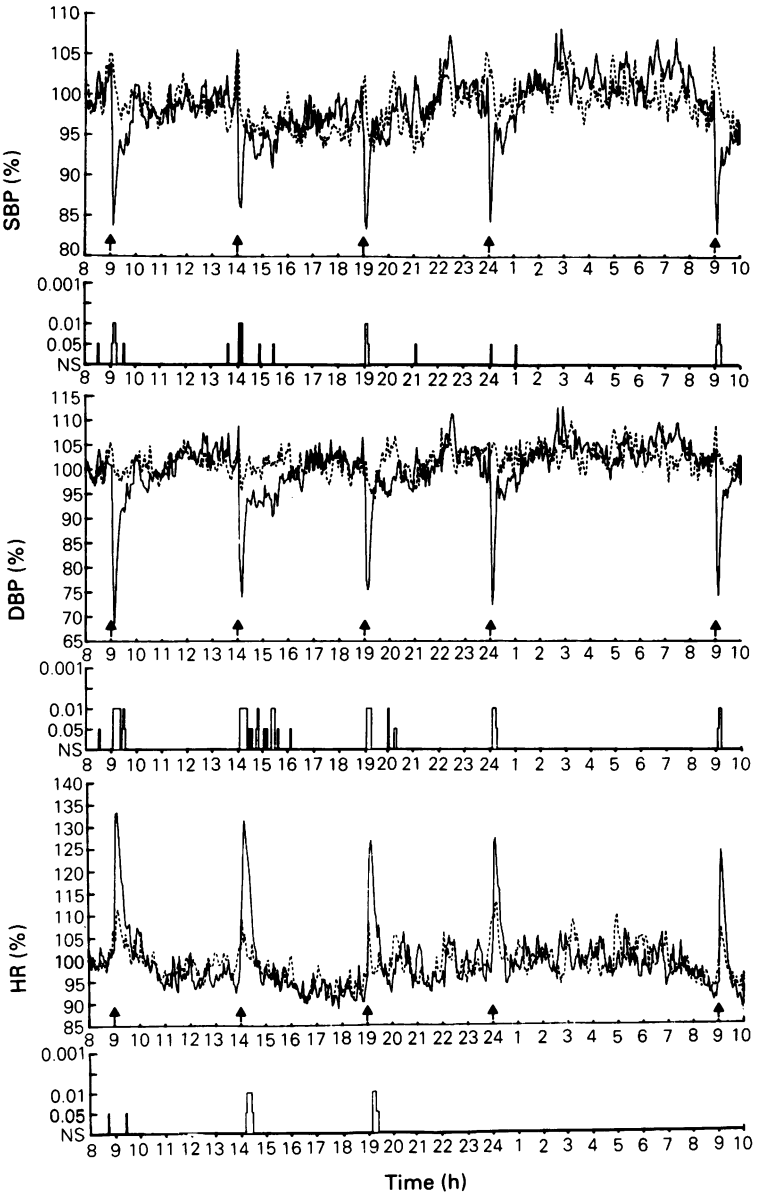


Figure 3 Chronograms of mean systolic blood pressure (SBP), diastolic BP (DBP) and heart rate (HR) recorded in spontaneously hypertensive rats receiving 5 injections of PY 108-068 (200 $\mu g\,kg^{-1}$, i.v.). Each point is the mean of the values recorded during 3 min periods. Arrows show the injection time. The lower panels in each pair indicate the statistical differences observed at each moment between treated ($n = 5$; —) and control ($n = 6$; – –) rats.

and remained significant for 20 min for SBP, 30 min for DBP and only 10 min for HR.

When considering PN 200-110, chronograms (see Figure 5) clearly showed that its hypotensive and tachycardiac actions were as rapid but more marked and longer lasting than those of PY 108-068. As well as for PY 108-068, PN 200-110 effects on BP appeared to be biphasic, made up of a rapid and marked decrease followed by a longer and weaker one. At a dose of 200 $\mu g\,kg^{-1}$, PN 200-110 decreased SBP by 25% and

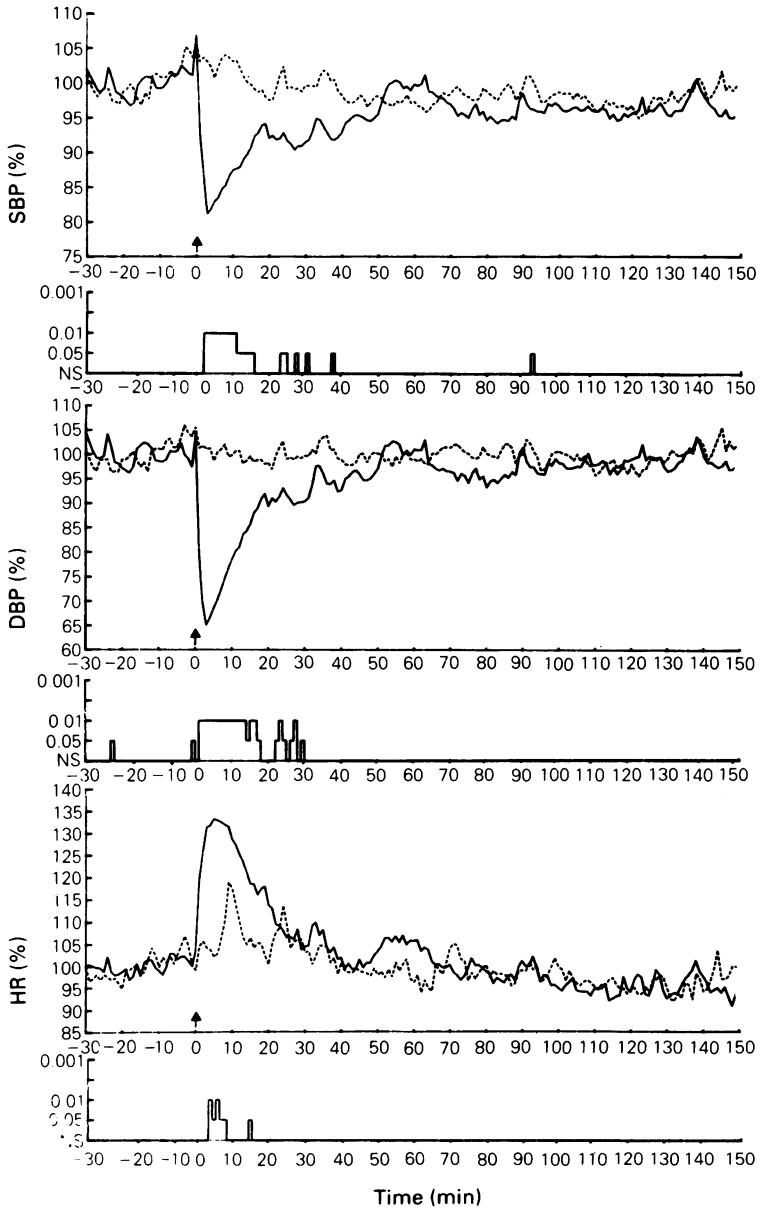


Figure 4 Chronograms of mean systolic blood pressure (SBP), diastolic BP (DBP) and heart rate (HR) in spontaneously hypertensive rats after the first injection of PY 108-068 (200 $\mu g\,kg^{-1}$, i.v.). Data are expressed as percentage of the values observed during the control period. Each point is the mean of the values recorded during 1 min. Arrows show the injection time. The lower panels in each pair indicate the statistical differences observed at each moment between treated ($n = 5$; —) and control ($n = 6$; – –) rats.

DBP by 45%. These BP effects remained significant for 3 h, as a mean, while the tachycardia lasted for 1 h only. When using smaller doses (50 and 100 $\mu g\,kg^{-1}$, i.v.) of PN 200-110, similar effects were observed but they were of shorter duration in a dose-related manner. This suggests that the vasodilator activity of PN 200-110 can only be manifest when its plasma concentrations are above a threshold level.

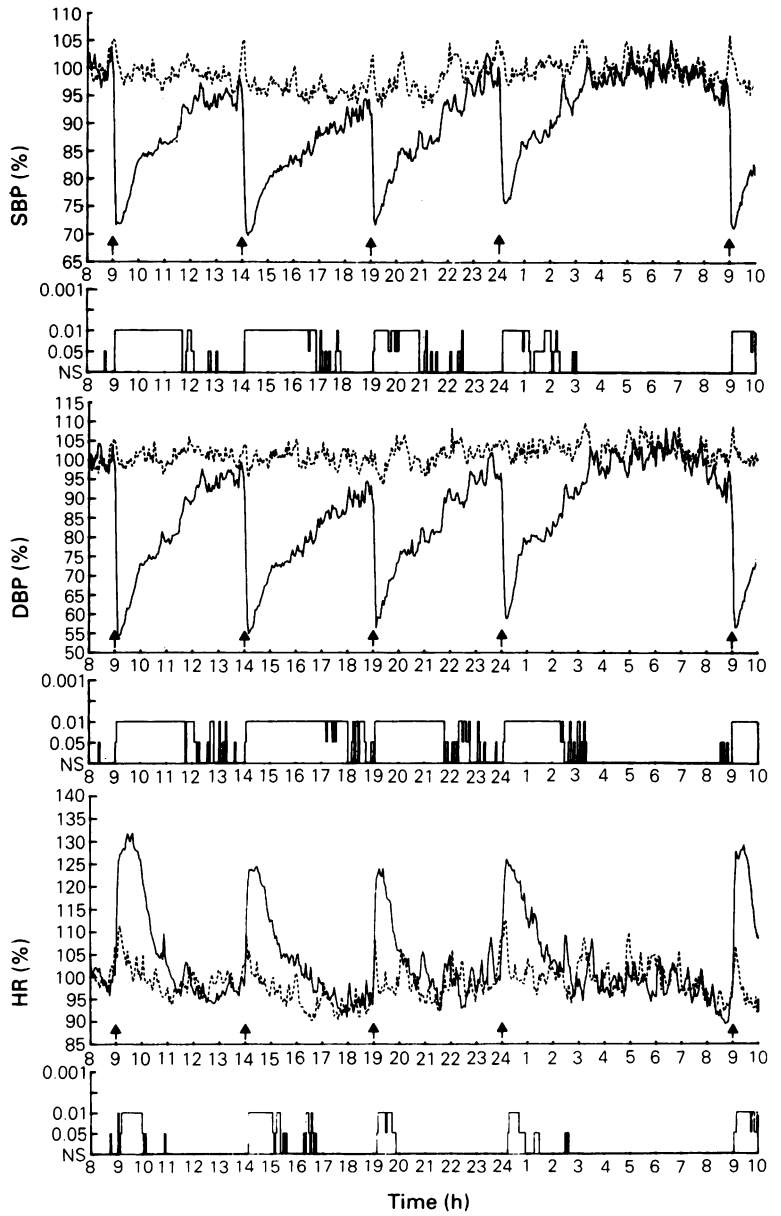


Figure 5 Chronograms of mean systolic blood pressure (SBP), diastolic BP (DBP) and heart rate (HR) recorded in spontaneously hypertensive rats receiving 5 injections of PN 200-110 (200 $\mu g\,kg^{-1}$, i.v.). Each point is the mean of the values recorded during 3 min periods. Arrows show the injection time. The lower panels in each pair indicate the statistical differences observed at each moment between treated ($n = 5$; —) and control ($n = 6$; – –) rats.

Baroreflex sensitivity

The baroreflex sensitivity was studied 1 h after the last 100 and 200 $\mu g\,kg^{-1}$, i.v. injections of drugs. As indicated in Table 2, PY 108-068 and PN 200-110 had no significant effect on the cardiac baroreflex response to phenylephrine and nitroglycerin bolus injections.

Table 2 Baroreflex sensitivity calculated after phenylephrine and nitroglycerin injections in control rats and in rats treated with PY 108-068 or PN 200-110

Drug ($\mu g\,kg^{-1}$)		*Baroreflex sensitivity* ($ms\,mmHg^{-1}$) *Phenylephrine*	*Nitroglycerin*
Controls	(6)	0.48 ± 0.06	0.88 ± 0.17
PY 108-068 100	(5)	0.46 ± 0.09	0.99 ± 0.14
PY 108-068 200	(4)	0.49 ± 0.07	1.33 ± 0.17
PN 200-110 100	(6)	0.67 ± 0.09	0.95 ± 0.11
PN 200-110 200	(5)	0.75 ± 0.17	1.39 ± 0.58

Values are given as mean ± s.e.mean. The number of animals studied is in parentheses.

Discussion

In the present work we studied the effects on BP and HR of repeated administration of different doses of PY 108-068 and PN 200-110, two new calcium entry blockers belonging to the dihydropyridine group. The SHR was the animal model used since it is known to be highly sensitive to the hypotensive action of dihydropyridines (Pegram *et al.*, 1984; Nagura *et al.*, 1986). The rats were studied while conscious and unrestrained in order to avoid the influence of anaesthesia or stress on the cardiovascular activity (Fluckiger *et al.*, 1985). Their aortic BP was continuously recorded and analysed for long periods of time by means of our previously described computerized technique (Cerutti *et al.*, 1985). This method allowed us to obtain not only SBP but also DBP which is of special interest in assessment of vasodilator effects. In addition, drugs could be injected into freely moving animals which were either resting (day-time) or active (night-time) which makes the rat model closer to the conditions of treated ambulatory patients. Finally, the software developed allowed us to adapt the time-basis used to average the data in order to describe precisely the time-course of the short-lasting changes in BP and HR induced by some calcium entry blockers.

The cardiovascular effects of PY 108-068 but not of PY 200-110 were previously described (Nievelstein *et al.*, 1985) in conscious SHR. Therefore, so as to facilitate the comparison between these 2 compounds, we decided to inject the same 3 doses of both. In these conditions, PY 108-068 induced almost immediate and dramatic falls in BP associated with increases in HR. The maximum changes in DBP were more marked than those in SBP, these reaching respectively −35% and −20% of their basal value after the dose of $200\,\mu g\,kg^{-1}$. It must be noted that such sharp changes were more precisely measured when the BP values were averaged each min than when they were averaged each 3 min as, in this latter case, the maximum decreases observed were found to be less (−30% and −17% for DBP and SBP respectively). The PY 108-068-induced changes in BP and HR were short-lasting (30 min for the highest dose used) and both their intensity and their duration were dose-related. These data are in close agreement with those of Nievelstein *et al.* (1985).

The cardiovascular effects of PN 200-110 were found to be qualitatively identical to those of PY 108-068 but they were greater and lasted for a much longer period of time (nearly 3 h). The BP decreases were still rapid, biphasic and more marked for DBP (−45%) than SBP (−25%). The lowest dose ($50\,\mu g\,kg^{-1}$) of PN 200-110 used induced nearly maximum effects. Taken together with its greater BP lowering action this finding suggests that, on a molar basis, PN 200-110 is more potent than PY 108-068. With increasing doses, DBP remained almost unchanged while SBP and the tachycardia slightly decreased. Such a decrease in the tachycardiac response was not observed with PY 108-068 and could be attributed to the intrinsic negative chronotropic action of PN 200-110 (Hof *et al.*, 1984a,b). As previously shown with PY 108-068 in rabbits (Hof & Scholtysik, 1983), this effect requires higher doses than the vasodilatation and could therefore contribute to the limitation of the tachycardia.

The effects of repeated injections of PY 108-068 and PN 200-110 were studied in order to determine whether tachyphylaxis developed in the BP or, more particularly the HR responses. As a whole, 5 injections of PY 108-068 or PN 200-110 given over 24 h, induced reproducible maximum effects.

Finally, the sensitivity of the cardiac baroreflex was carefully assessed in freely moving animals, which is of special importance in the measurement of reflex mediated responses (Fluckiger *et al.*, 1985). It was demonstrated that, 1 h after the 5th injection of either 100 or $200\,\mu g\,kg^{-1}$ of PY 108-068 and PN 200-110, the cardiac baroreflex response was increased by both drugs but not significantly.

In conclusion, the present work demonstrates that, in conscious SHR, both PY 108-068 and PN 200-110 induced marked hypotensive and tachycardic effects. With these two compounds, a tachyphylaxis could not be observed after 5 administrations repeated over a

24 h period and the baroreflex sensitivity was not significantly altered by such an acute treatment. Since PN 200-110 was more effective and longer lasting than PY 108-068 it appeared more likely to be useful in human therapy. Finally, the computerized study of the BP curve in conscious freely moving rats was found to be specially suitable for determination of the nature and the kinetics of the effects of new drugs acting on the cardiovascular system.

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