Br. J. Pharmac. (1987), **91**, 711–714

Increase of renal sympathetic nerve activity by metoprolol or propranolol in conscious spontaneously hypertensive rats

Stanislaw Majcherczyk, Andrzej Mikulski, May Sjölander & *Peter Thorén

Department of Pharmacology and Biochemistry, Hässle Cardiovascular Research Laboratories, S-431 83 Mölndal, Sweden and *Department of Physiology, University of Göteborg, S-400 33 Göteborg, Sweden

1 Mean arterial pressure (MAP), heart rate (HR) and renal sympathetic nerve activity (RSNA) were recorded in conscious spontaneously hypertensive rats (SHR).

2 Infusion of metoprolol ($4\,\mu mol\,kg^{-1}\,h^{-1}$) or propranolol ($1.5\,\mu mol\,kg^{-1}\,h^{-1}$) reduced HR and significantly increased RSNA.

3 Administration of metoprolol caused a sustained decrease of MAP starting in the third hour of infusion. In contrast, administration of propranolol induced a biphasic response in MAP. It is suggested that the increase of RSNA after both β-adrenoceptor blocking drugs is due to a decrease in arterial baroreceptor activity.

Introduction

Different modes of action have been suggested to explain the antihypertensive effects of β-adrenoceptor antagonists, including inhibition of sympathetic outflow to peripheral blood vessels (see Wallin *et al.*, 1984; van Baak *et al.*, 1985). Depending on the experimental method and set-up and the dose of the β-adrenoceptor antagonist, both inhibitory and excitatory responses have been obtained. The laboratory animals used have been either normotensive or hypertensive. Furthermore, a number of studies have been carried out under anaesthesia and different types of anaesthetics have been used (see Korner, 1982).

The aim of this study was to examine in detail sympathetic responses to β-blockers. By using a model with instrumented conscious spontaneously hypertensive rats, we could follow changes in sympathetic outflow in undisturbed rats. Our main finding is that the delayed decrease in arterial blood pressure is paralleled by an increase in renal sympathetic nerve activity.

Methods

Twenty-two male spontaneously hypertensive rats (SHR) of the Okamoto Aoki strain were used. One day before the experiment, the rats were anaesthetized with methohexitone sodium ($60-70\,mg\,kg^{-1}$ i.p.) and the renal sympathetic nerve branch was exposed via a retroperitoneal flank incision. A thin, bipolar electrode was placed around the nerve and insulated with silicone rubber (Wacker Sil Gel 604). Catheters were inserted into the left common carotid artery and into the right jugular vein. The catheters and the electrode cable were exteriorised on the neck. After surgery, the rats were allowed to recover for at least 16 h. During that period, all rats were connected to a swivel system and perfused with 1 cc per h of Ringer solution through the arterial cannula. The next day, the rats were placed in a covered perspex tube, wide enough to allow movements backwards and forwards. Basal heart rates before administration of drugs were normal (around 400 beats min^{-1}). The rats thus showed no evidence of stress at rest. The nerve signal was amplified (Grass P511) and rectified. The mean renal sympathetic nerve activity (RSNA), together with mean arterial pressure (MAP) and heart rate (HR), were continuously sampled by computer as described earlier (Lundin *et al.*, 1984). RSNA was expressed as % of its own basal level (100%).

After a stabilising period of 1–2 h, baseline values of MAP, HR and RSNA were established. After a control recording (60 min) of MAP, HR and RSNA, an intravenous bolus dose of metoprolol ($4\,\mu mol\,kg^{-1}$) or propranolol ($1.5\,\mu mol\,kg^{-1}$) was injected over 3 min. This was followed by a 4 h period of continuous infusion of metoprolol ($4\,\mu mol\,kg^{-1}\,h^{-1}$) or propran-

olol ($1.5\,\mu mol\,kg^{-1}\,h^{-1}$). In control experiments, equivalent volumes of Ringer solution were given. Arterial blood samples (for measurement of plasma concentrations of drugs) were taken at the end of each intravenous infusion. The *post-mortem* nerve activity was recorded in all animals for 30 min to measure the noise level; this was subsequently subtracted from the recorded value of the RSNA. Student's *t* test was used for statistical evaluation. A *P* value less than 0.05 was considered statistically significant. Values given are means ± s.e.mean.

Results

Metoprolol and propranolol reduced the HR to the same extent (Figure 1). The effect appeared immediately after the first bolus dose was given and persisted during the 4 h of drug infusion. However metoprolol and propranolol had different effects on MAP. Metoprolol caused a delayed decrease in MAP. Compared to values obtained in control SHR, the metoprolol-treated SHR showed a significant MAP reduction after 2 h (−8.2 ± 2.9 mmHg) and this effect persisted throughout the experiment. After propranolol, two distinct phases in the effects on MAP could be seen. During the first hour of infusion, a transient increase in MAP was observed (compared to appropriate controls). However, after 2 h MAP began to decrease and was significantly ($P<0.05$) lower in the third hour of infusion (Figure 1).

Both β-adrenoceptor antagonists produced statistically significant ($P<0.05$ propranolol, $P<0.01$ metoprolol) increases in RSNA in the second hour of infusion (metoprolol 13.9 ± 6.4%; propranolol 18.0 ± 12.1%) and this effect persisted until the end of the experiment (see Figure 1). The plasma concentrations of the administered drugs, obtained from blood samples, taken at the end of the experiments, ranged from 494 to 840 nmol l^{-1} for propranolol and from 374 to 680 nmol l^{-1} for metoprolol. These values correspond to the therapeutic range for β-adrenoceptor blockade in man (Edvardsson *et al.*, 1978).

Discussion

Our study clearly established that in conscious SHR metoprolol and propranolol produced prolonged increases in RSNA after a delay of about one hour. The advantages of the design of this study compared to those previously published are that we used conscious, hypertensive rats, an animal model with many similarities to essential hypertension in man (Folkow, 1983). In addition, the nerve recordings were performed on rats in good fluid balance one day after surgery, thus avoiding a blunting effect of anaesthesia

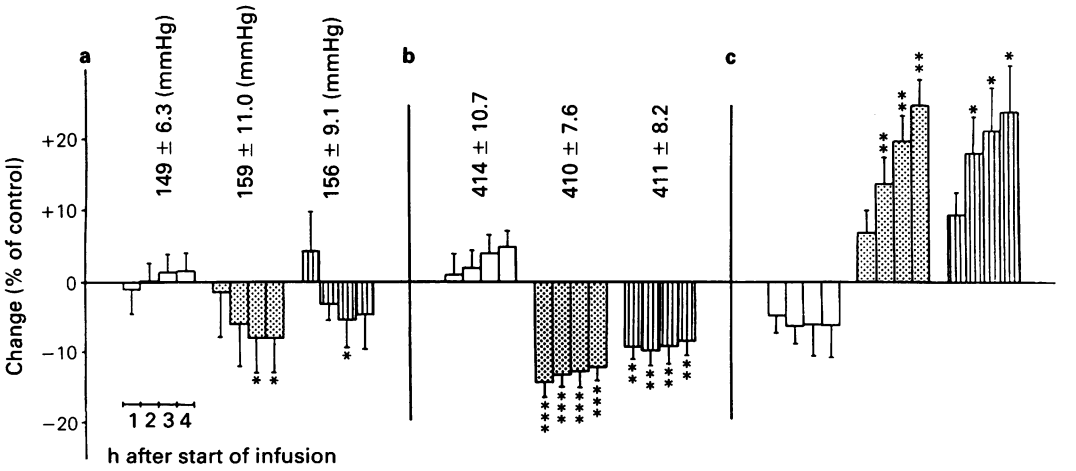

Figure 1 Effects of intravenous infusions of Ringer solution, metoprolol ($4\,\mu mol\,kg^{-1}\,h^{-1}$) and propranolol ($1.5\,\mu mol\,kg^{-1}$), for 4 h, on (a) blood pressure, (b) heart rate and (c) renal sympathetic nerve activity. The columns represent changes from basal level expressed as % of control period; vertical lines represent s.e.mean. Open columns represent control animals infused with Ringer solution ($n = 9$ rats); stippled columns, animals infused with metoprolol ($n = 7$ rats); and hatched columns, animals infused with propranolol ($n = 6$ rats). Values of arterial blood pressure and heart rate (beats min^{-1}) are shown above each group of columns (values are mean ± s.e.mean). Significance of the differences from control period: $*P<0.05$, $**P<0.01$, $***P<0.001$.

on cardiovascular reflexes. Finally, we also measured the plasma concentrations of metoprolol and propranolol, to ensure that the plasma levels were within the therapeutic range.

The immediate response to administration of either drug was a decrease in HR not accompanied by changes in MAP or RSNA. One hour later, however, we observed a significant rise in RSNA despite no significant change in MAP. In the third and fourth hour, we observed a significant decrease in MAP, paralleled by a further increase in RSNA.

The fact that the administration of either drug in this study did not cause an acute change in RSNA is surprising because injection of β-adrenoceptor antagonists in rats induces an immediate and marked decrease in cardiac output (van Baak *et al.*, 1985). This means that arterial pressure is maintained by perpheral vasoconstriction, probably neurally mediated and most likely due to increased sympathetic outflow in some other vascular areas. Our data thus differ from those obtained from experiments done in man by Sundlöf *et al.* (1983) and Wallin *et al.* (1984), who observed an immediate sympathetic excitation upon injection of metoprolol. One explanation for these discrepancies might be differences between species. Another explanation might be that Wallin *et al.* (1984) measured sympathetic activity in muscle nerves whereas we recorded renal nerve activity in our preparations.

Another interesting finding in this study was that the increase in RSNA in SHR preceded the antihypertensive effect of metoprolol. Since it is unlikely that a static unloading of arterial baroreceptors produced the initial increase in RSNA, other mechanisms should be considered. It is known, for example, that changes of dynamic blood pressure components can influence baroreceptor responses (Gero & Gerova, 1967; Angell-James & de Burgh Daly, 1970). One may speculate that the rapid reduction of HR induced by either drug could have produced dynamic unloading of the arterial baroreceptors (reduced dP/dt_{max}) and consequently induced the initial increase in RSNA. The delayed decrease in MAP after metoprolol could have additionally contributed to this effect by static unloading of the baroreceptors. This assumption is supported by the observation that only when baroreceptors were exposed to ambient arterial pressure was propranolol able to produce an increase in resistance in an isolated muscle bed (Lisander & Nilsson, 1978).

Another possible explanation for the increased RSNA could be that the β-adrenoceptor antagonists were acting directly on the central nervous system. It is likely, however, that the central action of such drugs has inhibitory effects on sympathetic drive and thereby contributes to the long term antihypertensive effects on these compounds, as shown both in hypertensive patients (Wallin *et al.*, 1984) and in SHR (Takeda & Bunag, 1980).

Previous investigations on the effect of various β-adrenoceptor antagonists on sympathetic activity have produced conflicting results (see Korner., 1982, van Baak *et al.*, 1985). It should be noted, however, that the animals used for those studies were either normotensive or hypertensive and either anaesthetized or conscious, and the reported effects were monitored for only 2 h after administration of the β-adrenoceptor blockers being tested. In addition, very few studies included measurement of the plasma level of the administered compounds. Our data can therefore not be compared with those from these earlier studies because we used direct nerve recordings in conscious unrestrained hypertensive rats.

In conclusion, direct nerve recordings of renal sympathetic activity in conscious spontaneously hypertensive rats show that the delayed decrease in arterial blood pressure observed two hours after continuous infusion of metoprolol or propranolol is accompanied by a rise in sympathetic activity. These results thus argue against the hypothesis that the antihypertensive effect of β-blockers is due to general sympathetic inhibition to all vascular areas. However, we do not exclude the possibility that the sympathetic drive to other vascular beds might decrease, as has been suggested for the muscle sympathetic outflow in man by Wallin and coworkers (1984).

References

ANGELL-JAMES, J.E. & de BURGH DALY, M. (1970). Comparison of the reflex vasomotor responses to separate and combined stimulation of carotid sinus and aortic arch baroreceptors by pulsative and non-pulsative pressure in the dog. *J. Physiol.*, **209**, 257–293.

EDVARDSSON, N., FRITZ, H., HALLEN, J., LARSSON, R., NORLANDER, S. & RASMUSSEN, P.A. (1978). Effect and plasma levels of metoprolol administered twice daily in essential hypertension. *Läkartidningen*, **75**, 1069–1072.

FOLKOW, B. (1983). Physiological aspects of primary hypertension. *Physiol. Rev.*, **62**, 347–504.

GERO, J. & GEROVA, M. (1967). Significance of the individual parameters of pulsating pressure in stimulation on baroreceptors. In: *Baroreceptors and Hypertension*, ed. Kezdi, P. pp. 17–30. Oxford: Pergamon.

KORNER, P.I. (1982). The mechanisms of action of β-adrenoceptor antagonists and the role of central and peripheral β-adrenoceptors in blood pressure regulation: A status report. In: *Trends in Autonomic Pharmacology*, Vol 2, ed. Kalsner, S. pp. 441–456. Baltimore, Munich: Urban & Schwarcenberg.

LUNDIN, S., RICKSTEN, S.E. & THOREN, P. (1984). Interac-

tion between 'mental stress' and baroreceptor reflexes concerning effects on heart rate, mean arterial pressure and renal sympathetic activity in conscious spontaneously hypertensive rats. *Acta physiol, scand.*, **120,** 273–281.

LISANDER, B. & NILSSON, H. (1978). Baro-receptor-induced decrease in muscle blood flow upon propranolol administration. *Eur. J. Pharmac.*, **50,** 275–278.

SUNDLÖF, G., WALLIN, B.G., STROMGREN, E. & NERHED, C. (1983). Acute effects of metoprolol on muscle sympathetic activity in hypertensive man. *Hypertension*, **5,** 749–756.

TAKEDA, K. & BUNAG, R.D. (1980). Chronic propranolol treatment inhibits sympathetic nerve activity and keeps blood pressure from rising in spontaneously hypertensive rats. *Hypertension*, **1,** 228–235.

van BAAK, M.A., STRUYKER BOUDIER, H.A.J. & SMITS, J.F.M. (1985). Antihypertensive mechanisms of beta-adrenoceptor blockade: A review. *Clin. & Exp. Hypertension Theory & Practice*, **A7 (1),** 1–72.

WALLIN, G., SUNDLOF, G., STROMGREN, E. & ABERG, H. (1984). Sympathetic outflow to muscles during treatment of hypertension with metoprolol. *Hypertension*, **6,** 557–562.

(Received February 19, 1986.
Revised February 9, 1987).
Accepted February 16, 1987.)