1983b; Greenblatt et al 1983). Comparison of in-vitro hplc retention, based on the method described above, with in-vivo drug distribution both in animals and man indicates that hplc retention is at least as reliable an index of in-vivo distribution as is the octanol : buffer partition coefficient. The present report indicates that hplc retention can be used to quantitate lipophilicity of β-adrenoceptor antagonists. Hplc retention was highly correlated with the octanol : buffer partition coefficient and confirmed propranolol to be by far the most lipophilic, with sotalol and atenolol the least lipophilic.

Hplc retention has a further merit in that it does not require the use of radioactive material, and it is easily standardized for comparisons of relative lipophilicity among many drugs.

REFERENCES

Arendt, R. M., Greenblatt, D. J., deJong, R. H. (1983a) Clin. Research. 31: 626A

Arendt, R. M., Greenblatt, D. J., deJong, R. H., et al (1983b) J. Pharmacol. Exp. Ther. 227: 98–106

Deacon, C. S., Lennard, M. S., Bax, N. D. S., Woods, H. F., Tucker, G. T. (1981) Br. J. Clin. Pharmacol. 12: 429–431

Divoll, M., Greenblatt, D. J., Shader, R. I. (1982) Pharmacology 24: 261–266

Greenblatt, D. J., Arendt, R. M., Abernethy, D. R., et al. (1983) Br. J. Anaesth. 55: 985–989

Hammers, W. E., Meurs, G. J., De Ligny, C. L. (1982) J. Chromatogr. 247: 1–13

Jack, D. B. (1981) Br. J. Clin. Pharmacol. 11: 402–406

Leo, A., Hansch, C., Elkins, D. (1971) Chem. Rev. 71: 525–616

Taylor, E. A., Jefferson, D., Carroll, J. D., Turner, P. (1981) Br. J. Clin. Pharmacol. 12: 549–559

Wells, M. J. M., Clark, C. R., Patterson, R. M. (1981) J. Chromatogr. Sci. 19: 573–582

Woods, P. E., Robinson, M. L. (1980) J. Pharm. Pharmacol. 33: 172–173

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The effect of week-long infusion of propranolol, via an osmotic minipump, on blood pressure, heart rate and vascular responses in spontaneously hypertensive rats

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Propranolol was infused in SHR subcutaneously for 7 days at two concentrations (either 3·75 or 7·5 mg kg^{-1} day) via a minipump. Mean blood pressure and heart rate measured under pentobarbitone anaesthesia on day 7 after implantation showed a significant dose-dependent decrease in both propranolol-treated groups. In the low-dose propranolol-treated rats, there was no change in contractile responses to phenylephrine over controls. In rats receiving the higher dose of propranolol there was a significant increase in the response to phenylephrine. There was no change in the relaxation response of any of the groups to isoprenaline. The results indicate that propranolol, while lowering blood pressure and heart rate, is also modifying the α-receptor response of the vascular wall in the spontaneously hypertensive rat.

Some studies have suggested that β-adrenergic blocking drugs may lower arterial pressure by a central mechanism (Day & Roach 1974). Smits et al (1980a) concluded, however, that the antihypertensive effects of propranolol in spontaneously hypertensive rats (SHR) were not caused by an action of the drug in the central nervous system. Pritchard & Gillam (1969) postulated that the hypotensive effect resulted from a resetting of the baroreceptors. Other studies (Frohlich et al 1968; Buhler et al 1973) have suggested that reductions in cardiac output or renin release are the primary causes. Some in-vitro studies have indicated that a prejunctional block of sympathetic nerve activity may be produced by propranolol (Day et al 1968). Others (Eliash & Weinstock 1971) also reported that propranolol in low doses reduced contractions of the nictitating membrane in the cat.

Smits et al (1980b), using the Alzet minipump for chronic, controlled delivery of propranolol in the SHR, were able to show a decrease in both blood pressure and heart rate. We have recently reported (Sun & Hanig 1983a) that the contractile response of aortic rings to phenylephrine in untreated SHR is markedly lower than that of controls of the Wistar-Kyoto (WKY) strain. We have also shown that week-long exposure of the normal Holtzman rat to propranolol, via the minipump, is without effect on blood pressure, heart rate or vascular reactivity (Sun & Hanig 1983b). We therefore considered it relevant to investigate the effect of prolonged infusion of propranolol on in-vitro vascular responses to phenylephrine and isoprenaline in the SHR and to relate these to observed changes in blood pressure and heart rate. The objective of this approach was to determine whether changes in reactivity of the arterial wall play a significant role in the cardiovascular actions of propranolol.

Materials and methods

Male SHR (Tac(N)SHR, derived originally from NIH, Taconic Farms Inc., Germantown, N.Y., USA), age 5

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months, of 300–350 g mean 320 g, were used. One or two minipumps (batch no. 01959, Alza Corp., Palo Alto, CA, U.S.A.) were implanted subcutaneously, under halothane anaesthesia, caudal to the last rib of the left side of the rat. The particular osmotic minipump used consisted of a 0·229 ml collapsible reservoir that released its contents at a rate of 1·05 μl h^{-1} (for the batch used in this study) by a driving force exerted by the swelling of the osmotic substance surrounding the reservoir. In this study, control animals received a minipump filled with 0·1% ascorbic acid, whereas in two other groups of SHR, one or two minipumps were implanted which contained propranolol at a concentration of 50 mg ml^{-1} in 0·1% ascorbic acid, corresponding to a daily release of 3·75 or 7·5 mg kg^{-1} day.

Mean blood pressure and heart rate were measured on the 7th day after implantation, under sodium pentobarbitone anaesthesia (40 mg kg^{-1}), by cannulation of the caudal tail artery. Animals were then decapitated, and the aorta excised and freed of fat tissue. Isolated ring segments of descending aorta (3–4 mm long) were set up in-vitro for isometric recording of the response on a Grass Model 7 polygraph (using a force displacement transducer FT.03C) according to the method previously described (Sun & Hanig 1983a, b). All drugs were freshly prepared in 0·9% NaCl daily and concentrations were expressed as the final concentration in the bathing medium.

The drugs used were: isoprenaline HCl (isoproterenol hydrochloride, Park-Davis, Detroit, MI), (−)-phenylephrine hydrochloride (Sterling-Winthrop Research Institute, Rensselaer, NY) and propranolol hydrochloride (Ayerst Laboratories Inc., New York, NY).

The data were evaluated statistically by Student's *t*-test for unpaired samples. The 0·05 level of probability was accepted as significant.

Results

The week-long infusion of propranolol caused decreases in blood pressure and heart rate of SHR at both doses. These changes were significantly different statistically from those obtained in controls (Table 1).

Table 1. Effect of chronic infusion of propanolol on blood pressure and heart rate of spontaneously hypertensive rats.[1]

	Blood pressure (mm Hg)	Heart rate (beats min^{-1})
Control	162 ± 7 (3)	380 ± 10 (3)
Propranolol		
3·75 mg kg^{-1}	132 ± 10 (5)[2]	304 ± 14 (5)[2]
7·5 mg kg^{-1}	117 ± 12 (5)[2]	290 ± 21 (5)[2]

[1] Value = mean ± s.e.m.
[2] Statistically significant from control ($P \leq 0·05$).

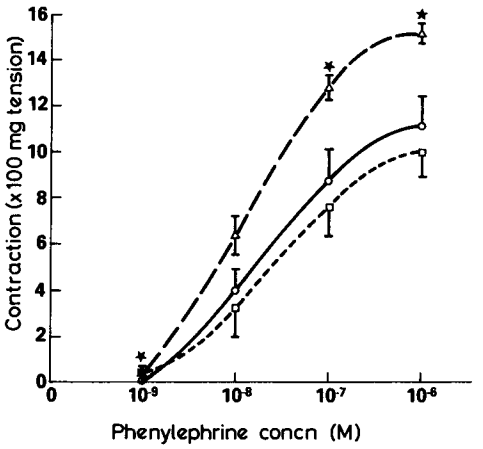

FIG. 1. Effect of pretreatment with propranolol for 7 days on vascular contraction. Values are means ± s.e.m.; n = 4. An asterisk indicates that the value is significantly different from the control values ($P < 0·05$). ——○—— control; - - - -□- - - - propranolol (3·75 mg kg^{-1} day); – –△– – propranolol (7·5 mg kg^{-1} day).

Fig. 1 shows the effect of pretreatment with propranolol on vascular contraction induced by phenylephrine. Pretreatment with 3·75 mg kg^{-1} day for 7 days did not change the contractile responses of the ring preparation to phenylephrine but 7·5 mg kg^{-1} day enhanced the contractile response with increases at all except one concentration of the α-agonist as well as a higher maximal response.

Pretreatment with either the high or the low dose of propranolol failed to produce a significant difference in vascular relaxation compared with untreated controls.

Discussion

A few investigators have shown that propranolol lowers blood pressure in the SHR (Garvey & Ram 1975; Sweets et al 1976). Others were unable to confirm this (Levy 1976; Numao & Irichijima 1974). As previously reported (Sun & Hanig 1983b), pretreatment with propranolol for one week, via a minipump, in normal Holtzman rats did not cause lowering of heart rate or blood pressure. In contrast, our present results indicate that continuous subcutaneous infusion of propranolol for one week in the unrestrained SHR causes increased vascular reactivity as well as a significant drop in both blood pressure and heart rate. The doses we used are comparable to those used by Smits et al (1980b) who reported cardiovascular effects in the intact SHR.

In another study (Sun & Hanig 1983a), we showed a statistically significant decrease in contractile responses to noradrenaline of aortic strips obtained from untreated SHR, when compared to WKY controls, confirming similar findings of Shibata & Kurahashi

(1972). We also found elevated levels of noradrenaline in aortic tissue of untreated SHR which led us to propose that α-receptor desensitization was playing an important role in the hypertensive process. It has also been postulated that diminished contractile response to α-adrenergic agonists in vasculature of SHR may involve morphological changes or an alteration of the geometrical structure in the resistance vessels (Folkow & Hallback 1977). These changes, which lead to an increase in total peripheral resistance, may also play a role in the etiology of hypertension.

If the normal Holtzman model pretreated with propranolol for one week is compared with the SHR, no change is observed in the former, whereas the latter shows a drop in both heart rate and blood pressure and a considerable increase in α-receptor reactivity. Therefore, we can say by inference that the normally desensitized α-receptors of the untreated SHR have been restored to a point of significantly greater reactivity concomitant with a drop in blood pressure and heart rate. The restoration of α-receptor reactivity by propranolol may have its origins presynaptically or possibly in the cns as evidenced by reduction of sympathetic tone (lowered blood pressure and heart rate) and presumably fewer numbers of noradrenergic agonist molecules that would be available to desensitize peripheral α-receptors. This is consistent with the work of Mukerjee & Lefkowitz (1977), which shows receptor desensitization with excess amounts of agonist and resensitization when the agonist stimulus is reduced. Propranolol caused no alteration in the function of the β-receptors in the SHR.

It is possible that dose and duration of propranolol treatment, as well as the characteristics of the in-vitro system may be contributing factors to the observed response and must be controlled for, and there is an additional possibility of concomitant changes in the connective tissue of the vascular wall that accompany the onset of hypertension in the SHR (Iwatsuki et al 1977; Andresen et al 1978) and that may be reversed in some fashion by pretreatment with propranolol.

In conclusion, our data show blood pressure and heart rate lowering effects of week-long propranolol exposure in SHR similar to those obtained in hypertensive patients, and these are concomitant with restoration of α-receptor reactivity.

REFERENCES

Andresen, M. C., Krauhs, J. M., Brown, A. M. (1978) Circ. Res. 43: 728–738

Buhler, F. R., Laragh, J. H., Vaughan, E. D., Brunner, H. R., Gavras, H., Baer, L. (1973) Am. J. Cardiol. 32: 511–522

Day, M. D., Roach, A. G. (1974) Clin. Exp. Pharmacol. Physiol. 1: 347–360

Day, M. D., Owenda, A., Warren, P. P. (1968) J. Pharm. Pharmacol. 20: Suppl. 130S–134S

Eliash, S., Weinstock, M. (1971) Br. J. Pharmacol. 43: 287–294

Folkow, B., Hallback, M. (1977) in: Genest, J., Koiw, E., Kuchel, O. (eds) Hypertension: Pathophysiology and Treatment. McGraw-Hill Book Co., New York, pp 507–529

Frohlich, E. D., Tarazi, R. C., Dustan, H. P., Page, I. H. (1968) Circulation 37: 417–423

Garvey, H. L., Ram, N. (1975) J. Pharmacol. Exp. Ther. 194: 220–233

Iwatsuki, K., Cardinal, G. J., Spector, S., Udenfriend, S. (1977) Science 198: 403–405

Levy, J. V. (1976) Am. J. Med. 61: 779–789

Mukerjee, C., Lefkowitz, R. J. (1977) Mol. Pharmacol. 13: 291–303

Numao, Y., Irichijima, Y. (1974) Jpn. Heart J. 15: 166–171

Pritchard, B. N. C., Gillam, P. M. S. (1969) Br. Med. J. 1: 7–16

Shibata, S., Kurahashi, K. (1972) in: Okamoto, K. (ed.) Spontaneous Hypertension: Its Pathogenesis and Complications. Igaku Shoin Ltd, Tokyo, pp 115–118

Smits, J. F. M., Van Essen, H., Struyker-Boudier, H. A. J. (1980a) J. Pharmacol. Exp. Ther. 215: 221–225

Smits, J. F. M., Van Essen, H., Struyker-Boudier, H. A. J. (1980b) J. Pharm. Pharmacol. 32: 139–140

Sun, C. J., Hanig, J. P. (1983a) Pharmacology in the press

Sun, C. J., Hanig, J. P. (1983b) Proc. Soc. Exp. Biol. Med. 172: 440–444

Sweets, C. S., Scriabine, A., Wenger, H. C., Ludden, C. (1976) in: Onesti, G., Fernandes, M., Stone, C. A. (eds) Regulation of Blood Pressure by the Central Nervous System, Green and Stratton, New York, pp 317–330