Pawłowski, L., Nowak, G. (1987) Biochemical and pharmacological tests for the prediction of ability of monoamine uptake blockers to inhibit the uptake of noradrenaline in-vivo: the effects of desipramine, maprotiline, femoxetine and citalopram. J. Pharm. Pharmacol. 39: 1003–1009

Pecknold, J. C., Ban, T. A. (1977) TRH in depressive illness. Int. Pharmacopsychiat. 12: 166–173

Sills, M. A., Jacobowitz, D. M. (1987) Chronic administration of desipramine or nialamide decreases wet-dog shakes in rats produced by the TRH-analog MK-771. Brain Res. 401: 195–199

J. Pharm. Pharmacol. 1989, 41: 641–643
Communicated January 24, 1989

Age-dependence of the effects of pinacidil on rat aorta

JANET C. WANSTALL, STELLA R. O'DONNELL, XIANG-PING ZENG, *Department of Physiology and Pharmacology, University of Queensland, St. Lucia, Brisbane, Queensland 4067, Australia*

Abstract—The effect of the K^+ channel opening drug, pinacidil, has been examined on aortic ring preparations from young (2 months) and aged (> 24 months) rats. The potency (neg log IC50) values for pinacidil in relaxing K^+ (20 mM)-contracted preparations were in the range expected for its K^+ channel opening (hyperpolarizing) effects but were not significantly different between young (6·34) and aged (6·31) rats. Thus, ageing does not affect the drug's potency as a K^+ channel opening drug. The more marked depression of the maximum response to noradrenaline by pinacidil (10 μM) in aged rats (85% reduction) compared with young rats (43% reduction), reflected a reduced α-adrenoceptor reserve for noradrenaline in preparations from aged rats. Pinacidil, in concentrations greater than 10 μM, was able to relax preparations contracted with 80 mM K^+ suggesting that it may have a second mechanism which does not involve hyperpolarization. It was more potent in producing this effect on the preparations from aged rats.

Vasodilator drugs are widely used in the treatment of hypertension and other cardiovascular disorders. Since these conditions are particularly common in the elderly, it is important to examine the influence of age on the responsiveness of blood vessels to vasodilator drugs. It has already been shown that ageing influences the in-vitro responses of blood vessels to different vasodilators, e.g. β-adrenoceptor agonists (Fleisch 1981; O'Donnell & Wanstall 1984, 1986), dopamine receptor agonists (Wanstall & O'Donnell 1988a) and calcium entry blocking drugs (Wanstall & O'Donnell 1988b, 1989), whilst having little or no effect on others, e.g. the nitrovasodilators (O'Donnell & Wanstall 1986; Wanstall & O'Donnell 1988a).

The aim of this study was to examine the influence of age on pinacidil, a vasodilator drug which has been suggested to have the novel mechanism of opening K^+ channels (Bray et al 1987). This drug has been examined on isolated aortic preparations from both young and aged rats. We have determined both its potency in relaxing K^+-induced contractions and its effects on concentration-response curves to noradrenaline.

Materials and methods

Male Wistar rats either 2 months old (young, 250–375 g) or 24–29 months old (aged, 440–530 g) were used.

Isolated single ring preparations (3 mm wide) of ventral aorta, from which the endothelium had been removed by gentle rubbing of the intimal surface, were set up in physiological salt solution (PSS) at 37°C, at a resting force of 10 mN, as described by Wanstall & O'Donnell (1988b). Force in the circular muscle was recorded isometrically, using a Statham Universal Transducer (UC3 and UL5).

After an initial 1h equilibration period, each preparation was contracted with K^+-depolarizing PSS (85·9 mM KCl) and relaxed with 100 μM 3-isobutyl-1-methylxanthine. After washout of this drug, one or two further contractions to K^+-depolarizing PSS were obtained. It has been found that this preliminary procedure results in preparations which have stable base-line tensions and which give consistent contractile responses for the duration of the experiment (Wanstall & O'Donnell 1988b, 1989).

Experimental protocols and expression of data. In the first series of experiments, cumulative concentration-response (contraction) curves to noradrenaline were obtained in the absence (control) and then in the presence of pinacidil (10 μM, contact time 30 min). Responses were expressed as a percentage of the maximum response to noradrenaline in the control curve so that any reduction in the maximum response could be determined. Control concentration-response curves to noradrenaline are reproducible in aorta from both young and aged rats (Wanstall & O'Donnell 1988b).

In the second series of experiments, preparations were contracted with KCl (20 or 80 mM—achieved by replacing the PSS with K^+-depolarizing PSS containing the appropriate KCl concentration). The contraction induced by 80 mM KCl was maximal, and that induced by 20 mM KCl submaximal (young 68%; aged 66% of the response to 80 mM KCl). In the absence of pinacidil, these contractions were sustained. Cumulative concentration-response (relaxation) curves to pinacidil (0·1 to 300 μM) were obtained once the spasmogenic response had reached equilibrium. Responses were expressed as % reversal of the induced contraction and were plotted against pinacidil concentration on a logarithmic scale. The concentration giving a 50% relaxant response (IC50) was interpolated and the negative log IC50 was used as an expression of relaxant potency.

Drugs and solutions. The drugs used were: 3-isobutyl-1-methylxanthine (Sigma); (−)-noradrenaline acid tartrate (Sigma) and pinacidil (gift from Leo Pharmaceuticals, Denmark). Stock solutions of noradrenaline (100 mM) and pinacidil (10 mM) were prepared in 10 mM HCl and of 3-isobutyl-1-methylxanthine (5 mM) in 10 mM NaOH. Dilutions of drugs were made in PSS.

The composition of the PSS was (mM): NaCl 118, KCl 5·9, $CaCl_2$ 1·5, $MgSO_4$ 0·72, glucose 11·7, ascorbic acid 1·14 (95% O_2/5% CO_2, pH 7·4). K^+-depolarizing PSS had the same composition as above, except that either 80 mM or 20 mM NaCl was replaced with 80 mM or 20 mM KCl (total KCl concentration = 85·9 mM or 25·9 mM).

Correspondence to: J. C. Wanstall, Department of Physiology and Pharmacology, University of Queensland, St. Lucia, Brisbane, Queensland, 4067 Australia.

Statistical analyses. Mean values are quoted together with their standard errors. The significance of differences was assessed by Student's *t*-test or by the Mann Whitney *U*-test.

Results

Effect of pinacidil on concentration-response curves to noradrenaline. On aorta rats of both age groups, pinacidil (10 μM) caused a non-parallel shift in the concentration-response curves to noradrenaline and a depression of the maximum responses (Fig. 1). The maximum response was depressed more on the preparations from aged rats ($85 \pm 2{\cdot}3\%$ reduction, n = 4) than on those from young rats ($43 \pm 8{\cdot}4\%$, n = 5, $P < 0{\cdot}05$, Mann Whitney *U*-test).

The preparations from the aged rats were less sensitive to noradrenaline than those from young rats (mean neg log EC50 values; aged $7{\cdot}81 \pm 0{\cdot}14$, n = 4; young $8{\cdot}38 \pm 0{\cdot}03$, n = 5; $0{\cdot}01 > P > 0{\cdot}001$, Student's *t*-test).

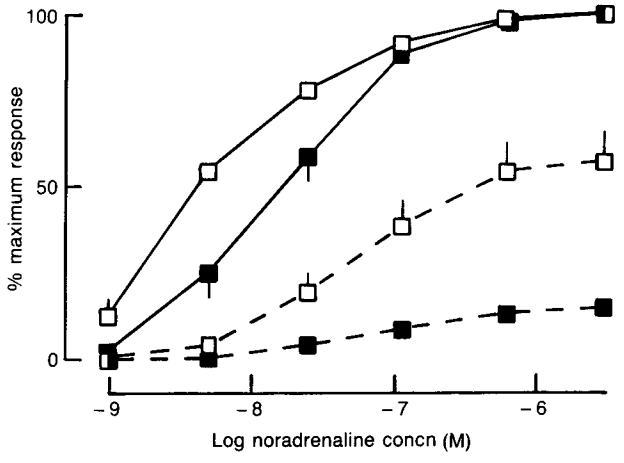

FIG. 1. Mean concentration-response curves to noradrenaline in the absence (controls, ——) and presence (- - -) of pinacidil (10 μM), on preparations of aorta from young (□, n = 5) and aged (■, n = 4) rats. Responses are expressed as % maximum response to noradrenaline in the control curves. Standard errors of the mean responses are shown except when smaller than the symbols.

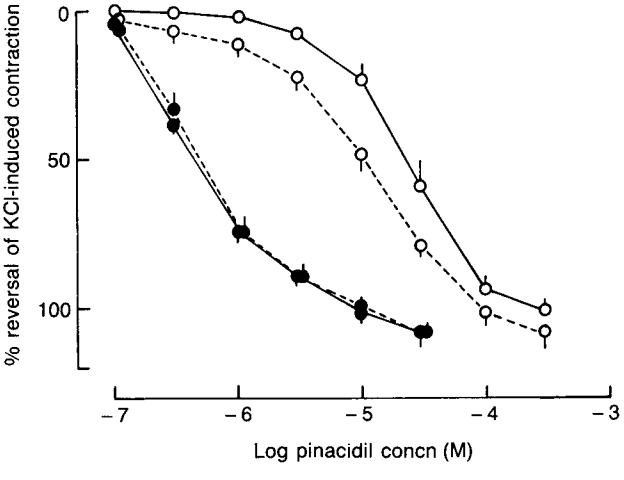

FIG. 2. Mean concentration-response (relaxation) curves to pinacidil on preparations of aorta from young (——) or aged (- - -) rats. The preparations were contracted with KCl 20 mM (●, n = 5) or 80 mM (○, n = 4). Responses are expressed as % reversal of the induced contraction. Standard errors of the mean responses are indicated except when smaller than the symbols.

Potency of pinacidil on K^+-contracted preparations of rat aorta. Pinacidil caused concentration-dependent relaxation of aortic preparations pre-contracted with 20 mM K^+ (Fig. 2). In preparations from both young and aged rats, complete reversal of the induced contraction was achieved with 10 μM pinacidil, and the negative log IC50 values were the same in both age groups (young, $6{\cdot}34 \pm 0{\cdot}04$, n = 5; aged $6{\cdot}31 \pm 0{\cdot}08$, n = 5; $P > 0{\cdot}05$, Student's *t*-test).

Pinacidil also relaxed preparations contracted with 80 mM K^+, but it was less potent against this higher concentration of K^+ (Fig. 2), and there was a small (2 fold) difference in potency between young and aged rats (mean negative log IC50 values, young $4{\cdot}64 \pm 0{\cdot}11$, n = 4; aged $4{\cdot}99 \pm 0{\cdot}09$, n = 4; $0{\cdot}05 > P > 0{\cdot}01$, Student's *t*-test).

Discussion

This study has shown that pinacidil (10 μM) caused more marked depression of contractions to noradrenaline in aortic preparations from aged rats than in those from young rats. This observation resembles previous findings with the calcium entry blocking drugs, diltiazem and felodipine (Wanstall & O'Donnell 1989) and can be explained if the mechanism of action of pinacidil is considered. The opening of K^+ channels by drugs such as pinacidil is associated with hyperpolarization of the cell membrane (Southerton et al 1988). Hyperpolarization can then (a) functionally antagonize depolarizing spasmogens by opposing the induced depolarization (Hof et al 1988) or (b) non-competitively antagonize non-depolarizing spasmogens by reducing the availability of Ca^{2+}, for contraction (Bray et al 1988). The mechanism(s) whereby the latter occurs remains to be elucidated, but could involve one or more of the following; the closing of voltage-operated Ca^{2+} channels, inhibition of the opening of receptor-operated channels (Videbaeck et al 1988), inhibition of Ca^{2+} entry through a Na^+/Ca^{2+} exchanger (Cook et al 1988) and/or increased uptake of Ca^{2+} into the sarcoplasmic reticulum (Erne & Hermsmeyer 1987).

Irrespective of whether pinacidil is acting as a functional or as a non-competitive antagonist of noradrenaline in rat aorta, a greater effect of pinacidil in aged rats can be predicted in light of the reduced α-adrenoceptor reserve for noradrenaline in these preparations with ageing. The reduced receptor reserve was established in a previous study on aortic tissues from aged rats (Wanstall & O'Donnell 1988b) and is reflected in the present study in the reduced potency of noradrenaline. According to theory, the smaller the receptor reserve for a spasmogen, the greater will be the depression in the maximum response to the spasmogen by a non-competitive antagonist or by a functional antagonist (van den Brink 1969, 1973).

On blood vessel preparations, including rat aorta, pinacidil has very little blocking effect on concentration-response curves to K^+ (Bray et al 1987; Wanstall & O'Donnell unpublished observations). Therefore, to quantify its effects on K^+-depolarized preparations in aorta from young and aged rats, its potency in relaxing K^+ pre-contracted preparations was determined, i.e. negative log IC50 values were obtained. On preparations contracted with 20 mM K^+ there was no difference in the potency of pinacidil between young and aged rats. This suggests that age does not influence the concentration range over which pinacidil causes hyperpolarization. Measurements of membrane potential would be required to confirm this conclusion.

Contractions to 80 mM K^+ are characteristically resistant to K^+ channel opening drugs because this concentration of K^+ ensures that the cell membrane remains depolarized regardless of whether the K^+ channels are open or not. Thus, in the present study, negligible relaxant responses were obtained on 80 mM K^+-contracted preparations when using concentrations of pina-

cidil which were effective against 20 mM K^+. Relaxation did, however, occur at higher concentrations of pinacidil (> 10 μM). This observation appeared to be contrary to the data of Weston et al (1988) and may indicate a secondary relaxant mechanism unrelated to the opening of K^+ channels. Furthermore, at these higher concentrations of pinacidil, there was an age-related increase in its potency, but this cannot be interpreted without knowing the mechanism involved in the postulated second action of pinacidil.

The main conclusions from this study of pinacidil on aorta from young and aged rats are that ageing appeared to have no influence on its potency as a hyperpolarizing agent and ageing increased its effectiveness against noradrenaline; this was predictable in view of the known reduction in the α-adrenoceptor reserve for noradrenaline in aorta from aged rats, and the non-competitive or functional nature of the antagonism between pinacidil and noradrenaline. Also, at high concentrations another vascular relaxant action may occur and this action may be influenced by age. The mechanism underlying this second action remains to be established.

The financial support of the National Health & Medical Research Council of Australia is gratefully acknowledged. JCW is an NH&MRC Research Scientist and XPZ an NH&MRC Visiting Overseas Scholar. We would like to thank Agatha Gambino for her excellent technical assistance and Dr Ian Ahnfelt-Rønne for the gift of pinacidil.

References

Bray, K. M., Newgreen, D. T., Small, R. C., Southerton, J. S., Taylor, S. G., Weir, S. W., Weston, A. H. (1987) Evidence that the mechanism of the inhibitory action of pinacidil in rat and guinea-pig smooth muscle differs from that of glyceryltrinitrate. Br. J. Pharmacol. 91: 421–429

Bray, K. M., Weston, A. H., McHarg, A. D., Newgreen, D. T., Southerton, J. S. (1988) Analysis of inhibitory action of BRL34915 on responses to noradrenaline in rabbit aorta. Ibid. 93: 206P

Cook, N. S., Weir, S. W., Danzeisen, M. C. (1988) Anti-vasoconstrictor effects of the K^+ channel opener cromakalim on the rabbit aorta—comparison with the calcium antagonist isradipine. Ibid. 95: 741–752

Erne, P., Hermsmeyer, K. (1987) Actions of pinacidil on calcium release in single vascular muscle cells of rat veins. The Physiologist 30: 129.

Fleisch, J. H. (1981) Age-related decrease in beta adrenoceptor activity of the cardiovascular system. Trends Pharmacol. Sci. 2: 337–339

Hof, R. P., Quast, U., Cook, N. S., Blarer, S. (1988) Mechanism of action and systemic and regional hemodynamics of the potassium channel activator BRL34915 and its enantiomers. Circ. Res. 62: 679–686

O'Donnell, S. R., Wanstall, J. C. (1984) Beta-1 and beta-2 adrenoceptor-mediated responses in preparations of pulmonary artery and aorta from young and aged rats. J. Pharmacol. Exp. Ther. 228: 733–738

O'Donnell, S. R., Wanstall, J. C. (1986) Thyroxine treatment of aged or young rats demonstrates that vascular responses mediated by β-adrenoceptor subtypes can be differentially regulated. Br. J. Pharmacol. 88: 41–49

Southerton, J. S., Weston, A. H., Bray, K. M., Newgreen, D. T., Taylor, S. G. (1988) The potassium channel opening action of pinacidil; studies using biochemical, ion flux and microelectrode techniques. Naunyn-Schmied. Arch. Pharmacol. 338: 310–318

van den Brink, F. G. (1969) Implications of the model of metactoid interaction; the concept receptor reserve. In: Histamine and antihistamines. Molecular pharmacology structure-activity relations, gastric acid secretion. Drukkerij Gebr Janssen, N. V. Nijmegen pp 53–56

van den Brink, F. G. (1973) The model of functional interaction. I. Development and first check of a new model of functional synergism and antagonism. Eur. J. Pharmacol. 22: 270–278

Videbaeck, L. M., Aalkjaer, C., Mulvany, M. J. (1988) Pinacidil opens K^+-selective channels causing hyperpolarization and relaxation of noradrenaline contractions in rat mesenteric resistance vessels. Br. J. Pharmacol. 95: 103–108

Wanstall, J. C., O'Donnell, S. R. (1988a) Norepinephrine acts on vascular dopamine receptors in rat perfused mesentery: influence of age. The Pharmacologist 30: A84

Wanstall, J. C., O'Donnell, S. R. (1988b) Inhibition of norepinephrine contractions by diltiazem on aorta and pulmonary artery from young and aged rats: influence of alpha-adrenoceptor reserve. J. Pharmacol. Exp. Ther. 245: 1016–1020.

Wanstall, J. C., O'Donnell, S. R. (1989) Influence of age on calcium entry blocking drugs in rat aorta is spasmogen-dependent. Eur. J. Pharmacol. 159: 241–246

Weston, A. H., Southerton, J. S., Bray, K. M., Newgreen, D. T., Taylor, S. G. (1988) The mode of action of pinacidil and its analogs P1060 and P1368: results of studies in rat blood vessels. J. Cardiovasc. Pharmacol. 12 (Suppl. 2): S10–S16