J. Pharm. Pharmac., 1967, **19**, 667–673 Received April 10, 1967

Effect of desipramine on the depolarized isolated renal artery

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Depolarization of the isolated renal artery of the rat by a potassium-rich solution caused a triphasic response. The artery contracted submaximally then relaxed partially, this relaxation being followed by a slowly developing contracture. Desipramine reduced the height of the initial contraction, increased the relaxation and reduced the contracture. The latter action was antagonized by increasing the calcium ion concentration of the depolarizing solution. Calcium caused a contraction of the depolarized artery and the contraction was reduced by the drug. The results suggest that the relaxation of the depolarized artery produced by desipramine may be due to interference with the action of calcium in initiating and maintaining a contraction of the arterial smooth muscle.

IMIPRAMINE-like drugs are known to produce a dual effect on the adrenergic system. At low doses they increase the pharmacological response to exogenous or endogenous noradrenaline (Sigg, 1959; Thoenen, Hürlimann & Haefely, 1964; Ursillo & Jacobson, 1965; Bonaccorsi & Garattini, 1966; Bonaccorsi & Hrdina, 1966). At relatively high doses, however, imipramine and its congeners decrease adrenergic responses. This inhibition has also been observed on the isolated renal artery (Hrdina & Garattini, 1966).

To obtain information about the effect of desipramine on mechanisms affecting membrane permeability it was decided to investigate its effect on the depolarized vascular smooth muscle.

Experimental

METHODS

A constant flow technique, as previously described (Hrdina & Garattini, 1966; Hrdina, Bonaccorsi & Garattini, 1967) was used to perfuse the isolated renal artery of the rat. Contraction of the artery caused an increase in perfusion pressure and relaxation of the artery a fall in perfusion pressure.

Solutions of the following constitution (mmoles/litre) were used for perfusion. *Krebs-bicarbonate solution:* NaCl 118·0; KCl 5·6; $CaCl_2$ 2·5; $MgSO_4$ 0·55; $NaHCO_3$ 15·0; KH_2PO_4 0·9; glucose 5·5; final pH 7·4. *K_2SO_4 depolarizing solution:* K_2SO_4 126·0; KCl 5·6; $CaCl_2$ 2·5; $KHCO_3$ 3·6; glucose 5·5 with the final pH adjusted to 7·4 with KOH.

The perfusion solutions were gassed continuously with a mixture of oxygen 95% and carbon dioxide 5%. The isolated renal artery preparation was first perfused with the Krebs-bicarbonate solution. After 60 min equilibration, the basic perfusion fluid was exchanged for the K_2SO_4

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depolarizing solution. Noradrenaline was injected in a volume of 0·1 ml proximal to the artery. Other drugs were added to the perfusion solutions or introduced by microinfusion using a Braun motor-driven syringe, delivering 0·1 ml/min of drug solution. Each drug concentration was tested on at least four, but generally six, different renal arteries.

Drugs used were: (—)-noradrenaline bitartrate (Recordati); cocaine hydrochloride (C. Erba); desipramine hydrochloride (Geigy, S. A.); chlorpromazine sulphate (Farmitalia); phenoxybenzamine hydrochloride (Smith Kline & French); phentolamine hydrochloride (Regitin amp., Ciba). Drug concentrations are expressed as the molar concentration of the salt, except noradrenaline, which is expressed as base.

Results

The effect of the depolarizing solution on the isolated renal artery. Depolarization of the isolated renal artery of the rat by a potassium-rich solution caused a triphasic response. The artery contracted submaximally then relaxed partially, this relaxation being followed by a slowly developing contracture (Fig. 1). In the depolarized artery noradrenaline is still able to produce a contraction, but its effect is reduced slightly as shown by the shift of the dose-response line to the right (Fig. 2).

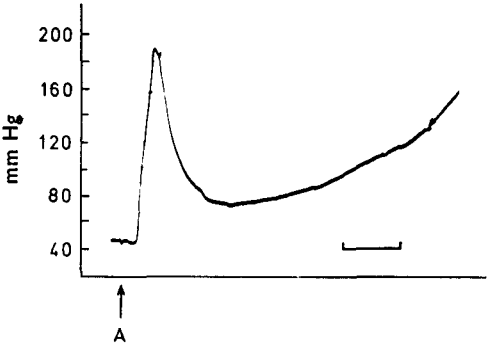

FIG. 1. Isolated perfused renal artery of the rat. At A, perfusion with ordinary Krebs bicarbonate solution was stopped and perfusion with K_2SO_4 depolarizing solution started. The response of the depolarizing solution has three phases (see text). Contraction of the artery is indicated by a rise in the perfusion pressure. Time scale: 10 min.

THE EFFECT OF DESIPRAMINE ON THE DEPOLARIZED ARTERY

The response of the artery to depolarization by K_2SO_4 Krebs solution was altered by desipramine which in a concentration of $6{\cdot}6 \times 10^{-6}$M reduced the initial contractile response to depolarization and increased the subsequent relaxation (Fig. 3). The reduction by desipramine ($6{\cdot}6 \times 10^{-8} - 6{\cdot}6 \times 10^{-6}$M) of the slow developing contracture to depolarization is also shown in Fig. 4.

The relaxant effect of desipramine on the slowly developing contracture following depolarization was quantitated by expressing the relaxation as

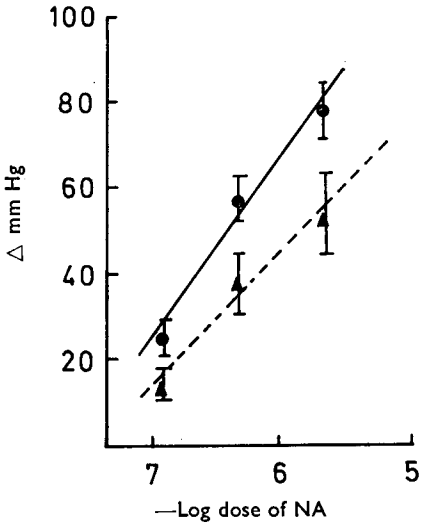

FIG. 2. The effect of K_2SO_4 depolarizing solution on the responses of the isolated renal artery to noradrenaline. Responses in Krebs-bicarbonate solution are shown by the continuous line and responses in the presence of K_2SO_4 depolarizing solution by the broken line. Each point with standard errors represents the mean of 4 experiments.

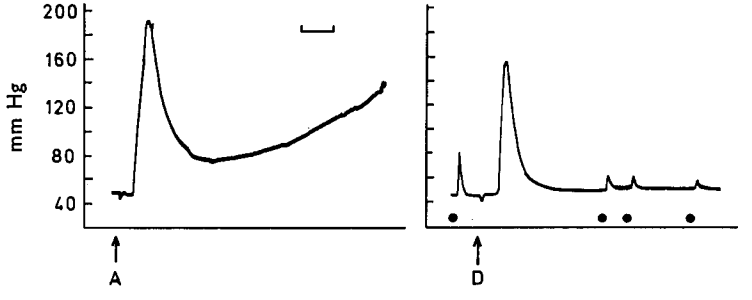

FIG. 3. Effect of desipramine on the response of isolated artery to K_2SO_4 depolarizing solution. Note the inhibition of the initial contraction and the following almost complete relaxation of muscle. The effect of a subsequent dose of noradrenaline (0·5 μg at ●) is diminished due to the adrenolytic action of desipramine at the concentration used. At A, K_2SO_4 solution; at D, K_2SO_4 solution + desipramine $6{\cdot}6 \times 10^{-6}$M. Time scale: 10 min.

a percentage of the maximal response of the artery to depolarization in the absence of the drug.

Desipramine in concentrations of $6{\cdot}6 \times 10^{-8}$, $1{\cdot}7 \times 10^{-6}$ and $6{\cdot}6 \times 10^{-6}$M produced a dose-dependent relaxation of the contracture response; these results are summarized in Table 1.

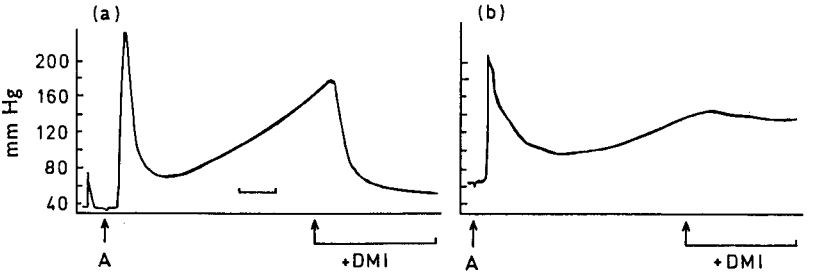

FIG. 4. Relaxing effect of two concentrations of desipramine (DMI) $6{\cdot}6 \times 10^{-6}$M in (a); $6{\cdot}6 \times 10^{-8}$M in (b) in the phase of the sustained contracture of the vessel. At A, K_2SO_4 solution alone. Time scale: 10 min.

THE EFFECT OF CHLORPROMAZINE, PHENTOLAMINE, PHENOXYBENZAMINE AND COCAINE

These drugs were tested on the contracture phase of the response to depolarization and the results were calculated as for desipramine above. Chlorpromazine ($5{\cdot}6 \times 10^{-6}$M) produced relaxations. Phenoxybenzamine ($5{\cdot}9 \times 10^{-6}$M) and phentolamine ($6{\cdot}25 \times 10^{-6}$M) had no effect. These results are summarized in Table 1. Cocaine (10^{-5}M) also had no effect on the contracture caused by depolarization however it induced relaxation at the high concentration of 4×10^{-4}M.

THE EFFECT OF DESIPRAMINE ON THE CONTRACTIONS CAUSED BY CALCIUM IN THE DEPOLARIZED ARTERY

Calcium ($6{\cdot}8 \times 10^{-5}$M) caused a contraction of the depolarized artery. The contraction was reduced by desipramine ($1{\cdot}7 \times 10^{-6}$M) (see Fig. 5). The reduction of the calcium induced contraction was proportional to the concentration of desipramine (Fig. 6). The depolarized artery contracted by potassium in the presence of calcium 2·5 mM was relaxed by desipramine ($6{\cdot}6 \times 10^{-6}$M) and this relaxation is converted to a contraction by increasing the concentration of calcium to 12·5 mM (Fig. 7).

Discussion

Several smooth muscle preparations when immersed in potassium-rich solutions respond by a characteristic contraction. Depolarized prepara-

TABLE 1. THE EFFECT OF DRUGS ON THE CONTRACTURE RESPONSE OF THE RENAL ARTERY TO DEPOLARIZATION BY K_2SO_4-KREBS SOLUTION

Drug	No. of experiments	Concn (M)	Relaxation* ±s.e. %
Desipramine	6	$6{\cdot}6 \times 10^{-8}$	2·38 ± 4·3
"	7	$1{\cdot}7 \times 10^{-6}$	36·1 ± 8·6
"	11	$6{\cdot}6 \times 10^{-6}$	51·8 ± 5·9
Chlorpromazine	5	$5{\cdot}6 \times 10^{-6}$	59·2 ± 14·9
Phentolamine	5	$6{\cdot}25 \times 10^{-6}$	8·1 ± 3·6
Phenoxybenzamine	4	$5{\cdot}9 \times 10^{-6}$	no effect

*Relaxation is calculated as the % of the maximal response that the tissue can produce in response to depolarization in the absence of the drug.

tions, including those of vascular muscle, still respond to various stimulatory or inhibitory drugs (Robertson, 1960; Durbin & Jenkinson, 1961; Edman & Schild, 1961; Briggs, 1962; Waugh, 1962a,b; Hinke & Wilson, 1962). However, there is a marked difference in the pattern of response to potassium-rich solutions of strips from a large conducting vessel (i.e. aorta) and from resistance vessels (i.e. artery of mesoappendix) (Bohr & Goulet, 1961). The contractile response of resistance vessels is rapid and is followed by a partial relaxation, while the response of aortic tissue is slower and sustained. The isolated perfused renal artery contracts in a similar manner to the resistance vessels, being a "muscular" type of

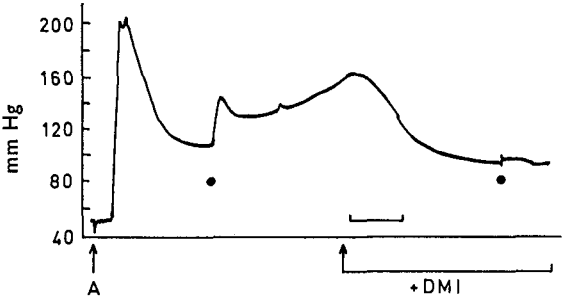

FIG. 5. The effect of calcium ions and desipramine on the depolarized renal artery. The artery was depolarized with K_2SO_4 at A. $CaCl_2$ ($6{\cdot}8 \times 10^{-5}$M at ●) caused a contraction which was reduced by desipramine ($1{\cdot}7 \times 10^{-6}$M). Time scale: 10 min.

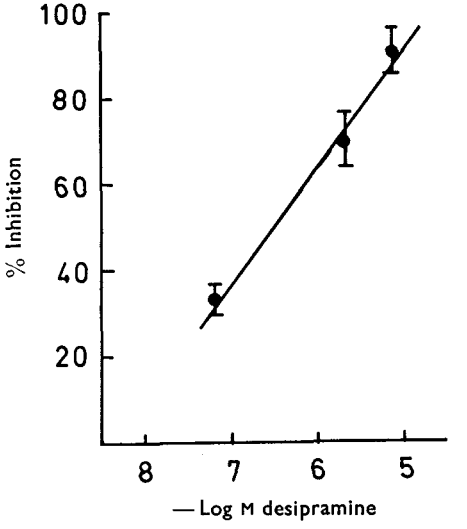

FIG. 6. The effect of desipramine on the contraction of the depolarized artery produced by calcium chloride ($6{\cdot}8 \times 10^{-5}$M). The reduction of the contraction by desipramine is expressed as a % of the initial contraction. Each point is the mean ± standard error of 4 experiments.

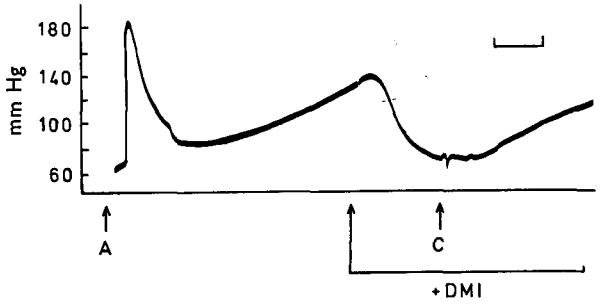

FIG. 7. The interaction of desipramine and calcium ions on the depolarized renal artery. The artery was depolarized with K_2SO_4 at A. At the second arrow desipramine $6{\cdot}6 \times 10^{-6}$M was added to the perfusate. At C, the calcium concentration, as $CaCl_2$, was raised from the normal level of 2·5 to 12·5 mM. Time scale: 10 min.

artery with an important function in regulating the blood flow into the kidney.

Noradrenaline still contracted the depolarized artery but its effect was slightly decreased. This is in accord with observations made by Cuthbert & Sutter (1965) and Jenkinson & Morton (1965) in other preparations. However, Hinke & Wilson (1962) did not observe any change in the effect of noradrenaline in the depolarized ventral tail artery of the rat.

Our results support the view based on previous findings (Haedings & Rondell, 1961; Waugh, 1962b; Cuthbert & Sutter, 1965; Kovalčik, Smyk & Blaškova, 1965), that depolarization of the membrane is not an essential step in the vasoconstriction induced by noradrenaline. Desipramine caused a decrease of the initial contractile response of vascular smooth muscle to the potassium-rich solution and also a dose-dependent relaxation of the muscle tone in the phase of the sustained contracture. It must be emphasized that even large doses of desipramine (10^{-5}M) do not change the muscle tone in a normal polarized vessel preparation.

Desipramine is known to have an adrenolytic effect (Haefely, Hürlimann & Thoenen, 1964; Ursillo & Jacobson, 1965) which has also been shown in preparations similar to those used in the present experiments (Bonaccorsi & Hrdina, 1966). However the drug was effective in relaxing the depolarized smooth muscle in a dose which did not exert an adrenolytic effect, but potentiated the response to noradrenaline. Phenoxybenzamine even in a high concentration ($5{\cdot}9 \times 10^{-6}$M) did not relax the depolarized preparation and phentolamine had much less effect than desipramine in a similar concentration. It does not seem likely that the reduction of the smooth muscle tone in depolarized artery induced by desipramine can be due to adrenolytic action. Furthermore there is no conclusive evidence that the contractile response of the smooth muscle to a potassium-rich depolarizing solution is due to stimulation of α-adrenergic receptors or to release of adrenergic transmitter, although such an effect has been suggested by Cervoni (1966).

The contractile response of smooth muscle to potassium-rich solution is attributed to an increased calcium influx due to changed membrane

permeability caused by potassium. The contraction was shown to be proportional to the concentration of calcium ions in the perfusate (Briggs, 1962; Waugh, 1962b; Hinke, Wilson & Burnham, 1964). Calcium itself is effective in causing contraction of depolarized smooth muscle, in contrast to its lack of action in normal preparations. The contraction of the depolarized renal artery caused by calcium was antagonized by desipramine to a degree proportional to the dose. On the other hand, an increase of calcium concentration in the perfusate increased the tone of relaxed muscle even in the presence of desipramine.

The drug and calcium can be considered therefore as functional antagonists, perhaps because desipramine, by stabilizing the membrane, inhibits the influx of calcium ions needed to trigger the initial contraction. The drug may also block the release inward of ionized calcium from the membrane. It is postulated that this release of calcium is responsible for the maintenance of the sustained contracture (Briggs, 1962; Hurwitz, Battle & Weiss, 1962); cocaine is similarly antagonized by calcium on the longitudinal muscle of the guinea-pig ileum (Hurwitz & others, 1962; Hagen & Hurwitz, 1963; Weiss, Coalson & Hurwitz, 1961).

In our experiments cocaine was able to reduce the tone of the depolarized artery but only in a concentration several hundred times higher than the effective concentration of desipramine. Chlorpromazine resembles desipramine in causing relaxation of the depolarized renal arteries. Chlorpromazine and desipramine are in this respect different from the potent adrenolytics phentolamine and phenoxybenzamine.

Acknowledgement. This work was partially supported by a grant from J. R. Geigy, S.A., Basel, Switzerland.

References

Bohr, D. F. & Goulet P. L. (1961). *Am. J. Cardiol.*, **8**, 549–556.
Bonaccorsi, A. & Garattini, S. (1966). *J. Pharm. Pharmac.*, **18**, 443–448.
Bonaccorsi, A. & Hrdina, P. (1966). *Symposium on Antidepressant Drugs*, Excerpta Medica Foundation, Amsterdam, Congress Series No. 122, 149–157.
Briggs, A. H. (1962). *Am. J. Physiol.*, **203**, 849–852.
Cervoni, P. (1966). *Fedn Proc. Fedn Am. Socs exp. Biol.*, **25**, 351.
Cuthbert, A. W. & Sutter M. C. (1965). *Br. J. Pharmac. Chemother.*, **25**, 592–601.
Durbin, R. P. & Jenkinson, D. H. (1961). *J. Physiol., Lond.*, **157**, 90–96.
Edman, K. A. P. & Schild, H. O. (1962). *Ibid.*, **161**, 424–441.
Haedings, V. E. & Rondell, P. A. (1961). *Physiologist*, **4**, 45.
Haefely, W., Hürlimann, A. & Thoenen, H. (1964). *Helv. physiol. Acta*, **22**, 15.
Hagen, S. V. & Hurwitz, L. (1963). *Fedn Proc. Fedn Am. Socs exp. Biol.*, **22**, 309.
Hinke, J. A. M. & Wilson, M. L. (1962). *Am. J. Physiol.*, **203**, 1161–1166.
Hinke, J. A. M., Wilson, M. L. & Burnham, S. C. (1964). *Ibid.*, **206**, 211–217.
Hrdina, P., Bonaccorsi, A. & Garattini, S. (1967). *Europ. J. Pharmac.*, **1**, 99–108.
Hrdina, P. & Garattini, S. (1966). *J. Pharm. Pharmac.*, **18**, 259–260.
Hurwitz, L., Battle, F. & Weiss, G. B. (1962). *J. gen. Physiol.*, **46**, 315–332.
Jenkinson D. H. & Morton, I. K. M. (1965). *Nature, Lond.*, **205**, 505–506.
Kovalčik, V., Smyk, L. & Blaškova, I. (1965). *Cs. Fysiol.*, **14**, 297–298.
Robertson, P. A. (1960). *Nature, Lond.*, **186**, 316.
Sigg, E. B. (1959). *Can. Psychiat. Ass. J.*, **4**, S75–S85.
Thoenen, H., Hürlimann, A. & Haefely, W. (1964). *J. Pharmac. exp. Ther.*, **144**, 405–414.
Ursillo, R. C. & Jacobson, J. (1965). *Ibid.*, **148**, 247–251.
Waugh, W. (1962a). *Circulation Res.*, **11**, 264–276.
Waugh, W. (1962b). *Ibid.*, **11**, 927–940.
Weiss, G. B., Coalson, R. E. & Hurwitz, L. (1961). *Am. J. Physiol.*, **200**, 789–793.