5% CO_2. The resulting pH was 7.3. Contractions of the muscle were recorded isometrically by means of a strain gauge ('Grass' Force Displacement transducer FT-O3C) connected to a 'Devices' heated stylus recorder. The muscle was maintained at a resting tension of 1 g.

Electrical stimulation was via a pair of open field electrodes running parallel to the muscle, and connected to a 'Grass' S88 stimulator. The stimulation parameters necessary to elicit responses varied considerably between preparations, but were usually of the order of 10 Hz, 10V, and 10 ms, applied for 5 s in fresh specimens. The muscle contracted rapidly to a voltage dependent maximum, but the peak amplitude of contraction was not sustained and relaxation, less rapid than the contraction, began before the stimulus had finished.

Permission to use the bladder specimens was obtained from The Middlesex Hospital Ethics Committee. Materials used were acetylcholine chloride (Koch-Light Laboratories) and dopamine hydrochloride (Sigma Chemical Company), and all concentrations are expressed as final concentrations in the organ bath.

Spontaneous activity, when present, was low, and graded responses to electrical stimulation and to acetylcholine (1–100 μmol/1) were obtained. Sensitivity to both types of stimulus was lower in stored specimens, usually by a factor of at least ten.

Dopamine in concentrations up to 1 mmol/1 did not elicit any contraction of the muscle, and was without effect upon its resting tone (n=7). When added in stepwise concentrations up to 5 mmol/1 during a sustained contraction produced by acetylcholine, dopamine did not modify the contraction. It was likewise without effect if added 1–20 min prior to the addition of acetylcholine.

Similarly, dopamine was without effect upon responses to electrical stimulation.

We can conclude from these findings that our experiments failed to provide evidence for dopaminergic receptors in the detrusor muscle of the human urinary bladder and that the findings with the dog bladder muscle preparations are not generally applicable.

P.F. HEFFRON
Department of Pharmacology & Therapeutics, The Middlesex Hospital Medical School, London W1P 7PN

E.J. G. MILROY
Department of Urology, The Middlesex Hospital, London W1P 8AA

Received June 27, 1980

References

BENSON, G.S., RAEZER, D.M., ANDERSON, J.R., SAUNDERS, C.D. & CORRIERE, J.N. (1976). Effect of levodopa on urinary bladder. *Urology*, **7**, 24–28.

FARRAR, D.J. & OSBORNE, J.L. (1976). The use of bromocriptine in the treatment of the unstable bladder. *Br. J. Urol.*, **48**, 235–238.

METCALFE, H.L. & TURNER, P. (1969). A comparison of the effects of cinanserin and methysergide on responses

ATTENUATION BY SQ 14,255 (CAPTOPRIL) OF THE VASCULAR RESPONSE TO NORADRENALINE IN THE RAT ISOLATED KIDNEY

In addition to inhibition of angiotensin I-converting enzyme (ACE) activity and potentiation of the hypotensive action of exogenous bradykinin, some observations suggest that SQ 14,255 may have other unknown effect(s). It has been shown that SQ 14,255 blunts the development of spontaneous hypertension in rats at the dose of 100 mg kg^{-1} day^{-1} which seems unequivocally higher than that needed for the sole inhibition of angiotensin II generation (Ferrone & Antonaccio, 1979). Recently, it was reported that this agent lowers supine arterial pressure and induces severe orthostatic hypotension only after substantial volume removal in anephric patients (Man In't Veld, Schicht, Derkx, De Bruyn & Schalekamp, 1980). Since overactivity of the sympathetic nervous system is present in spontaneously hypertensive rats (SHR) (Judy, Watanabe, Henry, Besch, Murphy and Hockel, 1976) and volume depletion, we assessed the influence of SQ 14,225 on the vascular responsiveness to noradrenaline (NA) and other vasoconstrictor substances (angiotensin II and 5-hydroxytryptamine (5-HT)) in the blood-free perfused rat isolated kidney.

Male Sprague-Dawley rats weighing 250 to 350 g and maintained on *ad libitum* sodium intake were anaesthetized with pentobarbital (50 mg/kg, i.p.) and heparinized (50–100 U, i. v.). The left kidney was

isolated and perfused without interruption of blood flow according to Vandongen, Peart & Boyd (1973). The perfusion medium consisted of Tyrode's solution containing 2% of dextran (molecular weight 70,000), gassed with a 95% O_2–5% CO_2 mixture and maintained at 37°C. The perfusion fluid was delivered at a constant flow of 5 ml/min with a Harvard peristaltic pump (model 1210). Venous effluent was discarded and perfusion pressure distal to the tip of the arterial cannula (PP) was continuously recorded with a Statham P 23 DB transducer. In such a system any change in PP directly reflects alteration in renal vascular resistance.

After stabilization of PP (usually within 30 min), experiments were started. The response of the system to bolus injections of NA (at doses of 20, 40 and 100 ng), angiotensin II (AT II at doses of 2.5, 5 and 10 ng) and 5-HT (at doses of 20, 40 and 100 ng) was assessed in three groups of 9, 7 and 7 rats respectively, before and during constant infusion into the arterial line of SQ 14,255 at doses of 100 and 500 μg/min. In six experiments the influence of similar doses of the nonapeptide converting enzyme inhibitor SQ 20,881 on the response to NA was assessed. In eight experiments, dose-response curves to NA were carried out before and during constant infusion of the vehicle (0.9% saline) in order to assess the stability of the response of the preparation to NA (time control studies). At the end of each experiment, we checked that complete blockade of angiotensin-converting enzyme activity by SQ 14,225 or SQ 20,881 was obtained by evaluating the response to a bolus injection of 200 ng of angiotensin I.

Results are expressed as mean ± s.e. mean and statistical analysis was carried out by the Student's *t*-test in which paired test was used to compare control and experimental responses in the same kidney.

PP of the isolated kidney was 70 to 90 mm Hg at the start of perfusion and stabilized at 30 ± 2 mm Hg within approximately 30 min. PP was unchanged during infusion of both ACE inhibitors or saline.

As shown in Figure 1, SQ 14,225 produced a dose-dependent shift to the right of the dose-response curve to NA. The vasoconstrictor response to 20, 40 and 100 ng of NA was lower than control by 24 ± 8, 35 ± 7 and 20 ± 7% respectively when SQ 14,225 was infused at a dose of 100 μg/min and by 46 ± 8, 60 ± 6 and 46 ± 8% respectively when SQ 14,225 was delivered at a dose of 500 μg/min. On the opposite, the vasoconstrictor response to NA was not significantly affected during infusion of SQ 20,881. In time control studies, the response to NA was not modified when saline instead of converting enzyme inhibitors was infused into the system.

Studies on the influence of SQ 14,225 on the response to AT II showed that only at the highest dose of SQ 14,225 the response to doses of the octapeptide of 2.5, 5 and 10 ng was significantly diminished by 24 ± 7, 20 ± 8 and 14 ± 6% respectively. The vasoconstrictor effect of 5-HT was not affected by SQ 14,225.

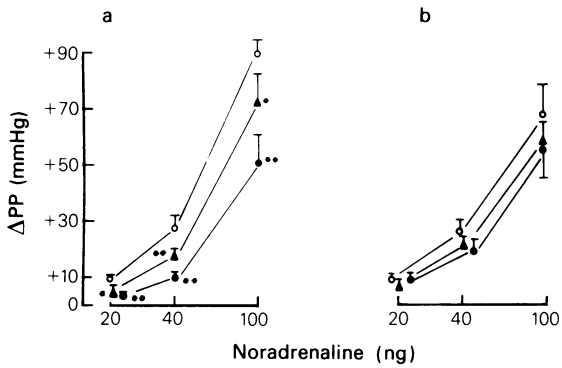

Figure 1 Influence of (a) SQ 14,225 and (b) SQ 20,881 on the increase in renal perfusion pressure (Δ PP) induced by bolus injections of noradrenaline (NA). The doses of converting enzyme inhibitors correspond to concentrations of 20 and 100 μg/ml in the perfusion medium. Results are expressed as mean ± s.e. mean and significance (paired *t*-test) is indicated by asterisks (* $P < 0.05$, ** $P < 0.005$). ○ control, ▲ SQ 14,225 or SQ 20,881 100 μg/min, ● SQ 14,225 or SQ 20,881 500 μg/min.

Bolus injection of 200 ng angiotensin I produced an increase in PP of 40 ± 7 mm Hg in studies in which converting enzyme inhibitors were not infused; no detectable vasoconstrictor action of angiotensin I was observed during infusion of either SQ 14,225 or SQ 20,881.

In the present studies, infusion into the arterial line of SQ 14,225 at doses of 100 and 500 μg/min corresponding to concentration of 20 and 100 μg/ml in the perfusion medium attenuated markedly and in a dose-dependent manner the vasoconstrictor response to NA. Since SQ 20,881 was ineffective, it is unlikely that inhibition of converting enzyme and its consequences on local generation of angiotensin II and survival of kinins could be responsible for this observation.

A similar attenuation of the action of NA by SQ 14,225 was reported by Okuno, Kondo, Konishi, Saruta & Kato (1979) in blood-free perfused rat mesenteric arteries; however, the perfusate concentration of SQ 14,225 associated with a 50% inhibition of the effect of NA was in the range of 10 μg/ml whilst it is close to 100 μg/ml in the present studies. Such observations are in contrast with previous studies in which high concentrations of SQ 14,225 had no effect on the vasoconstrictor response to NA of several isolated smooth muscle preparations (Rubin, Laffan, Kotler, O'Keefe, Demaio & Goldberg, 1978). The use of different smooth muscle preparations may explain these discrepancies.

While SQ 14,225 did not affect the response to 5-HT, it produced a slight but significant decrease in the response to angiotensin II, only when infused at the highest dose level. This could be related to inhibition by SQ 14,225 of the noradrenaline-dependent component of the vascular response to AT II which is known to stimulate the release of NA from sympathetic nerve endings (Chevillard, Duchene & Alexandre, 1971). Although our results are in favour of a specific interaction of SQ 14,225 with the vascular reactivity to NA, more studies are needed in order to elucidate the mechanism underlying this observation.

In conclusion, the present results may partly explain the preventive effect of high doses of SQ 14,225 on the development of hypertension in SHR and the hypotensive effect of a single 100 mg dose of the compound observed in anephric subjects only after substantial volume depletion, a manoeuvre which stimulates the activity of the adrenergic nervous system.

The authors are grateful for the gift of SQ 14,225 and SQ 20,881 to Dr Zola P. Horovitz (E.R. Squibb and Sons, Princeton, N.J., U.S.A.)

DANIEL CASELLAS, ALBERT MIMRAN, MADELEINE DUPONT & CLAUDE CHEVILLARD

Department of Medicine, Saint-Charles Hospital, Montpellier, France.

Received June 12, 1980

References

CHEVILLARD, C., DUCHENE, N. & ALEXANDRE, J.-M. (1971). Selective release of newly synthetized cardiac norepinephrine induced by angiotensin II. *Eur. J. Pharmac.*, **15**, 8–13.

FERRONE, R.A. & ANTONACCIO, M.J. (1979). Prevention of the development of spontaneous hypertension in rats by captopril (SQ 14,225). *Eur. J. Pharmac.*, **60**, 131–137.

JUDY, W.V., WATANABE, A.M., HENRY, D.P., BESCH, H.R., MURPHY, W.R. & HOCKEL, G.M. (1976). Sympathetic nerve activity. Role in regulation of blood pressure in the spontaneously hypertensive rat. *Circulation Res.*, **38** (suppl. 2) 21–29.

MAN IN'T VELD, A.J., SCHICHT, I.M., DERKX, F.H., DEBRUYN, J.H. & SCHALEKAMP, M. (1980). Effects of an angiotensin-converting enzyme inhibitor (captopril) on blood pressure in anephric subjects. *Br. med. J.*, **1**, 288–290.

OKUNO, T., KONDO, K., KONISHI, K., SARUTA, T. & KATO, E. (1979). SQ 14,225 attenuates the vascular response to norepinephrine in the rat mesenteric arteries. *Life Sci.*, **25**, 1343–1350.

RUBIN, B., LAFFAN, R.J., KOTLER, D.G., O'KEEFE, E.H., DEMAIO, D.A. & GOLDBERG, M.E. (1978). SQ 14,225 (D-3-mercapto-2-methylpropranoyl-L-proline), a novel orally active inhibitor of angiotensin I-converting enzyme. *J. Pharmac. exp. Ther.*, **204**, 271–280.

VANDONGEN, R., PEART, W.S. & BOYD, G.W. (1973). Adrenergic stimulation of renin secretion in the isolated perfused rat kidney. *Circulation Res.*, **32**, 290–296.

HAEMODYNAMIC EFFECTS OF DILTIAZEM IN HEALTHY VOLUNTEERS

Diltiazem (DTZ) is a new calcium antagonist (Cavero, Boudot, Lefevre-Borg & Roach, 1978). Two studies, double-blind and cross-over against placebo, have been carried out in two groups of eight healthy volunteers in order to assess its effects on the heart (chronotropic and inotropic actions), systemic pressure and venous compliance.

The aim of the first protocol was to determine the effects of DTZ on heart rate, blood pressure (cuff manometer), and venous compliance (mercury in rubber strain gauge placed around the lower part of the calf), in the supine and erect positions (tilt head-up 45°). Eight subjects (six females, two males, aged 22.7 ± 7 years, weight 62 ± 9.9 kg) were given a single dose of DTZ 120 mg or placebo p.o, with an interval of 7 days between the two trials, and measurements were performed before the administration of drug and every 30 min, up to 180 min there after. At the third hour four out of eight subjects performed a submaximal ergometric test (heart rate > 160/min). There was no significant variation (variance analysis) in blood pressure, heart rate, or venous compliance in supine or tilt positions. During exercise, heart rate, systemic blood pressure or double product (calculated as HR × systolic BP) did not change. The plasma level of DTZ (Rovei, Mitchard & Morselli, 1977), determined at the third hour, was 60.3 ± 42 ng/ml (range 17–144).

In the second protocol, eight other volunteers (five females, three males; aged 23.5 ± 9 years, weight 62.7 ± 9.9 kg) were given a single dose of DTZ 180 mg or placebo. Heart rate, blood pressure and systolic time intervals were recorded before and 3 h after taking the drug. PEP, LVET and QS_2 were corrected for