# Effects of the Nonapeptide SQ 20881 on Blood Pressure of Rats with Experimental Renovascular Hypertension (37348)

S. L. ENGEL, T. R. SCHAEFFER, M. H. WAUGH, AND B. RUBIN
*The Squibb Institute for Medical Research, Princeton, New Jersey 08540*

Earlier reports (1–3)[1] described the ability of the nonapeptide SQ 20881 (<Glu-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro) and related compounds to inhibit the pressor effects evoked by angiotensin I, but not those evoked by angiotensin II, in rats and dogs. This activity was attributed at least in part to an inhibition of the enzyme that converts the nonpressor peptide angiotensin I to its potent pressor derivative angiotensin II, an activity that had already been demonstrated *in vitro* (4, 5). These peptides also augment the vasodepressor activity of bradykinin (1, 6), as well as its contractile effect in isolated tissues (7), presumably by inhibiting kininase(s) that inactivate bradykinin (8). The present studies in rats were undertaken to determine the effects of SQ 20881 on blood pressure in four models of renovascular hypertension.

*Materials and Methods.* Studies were performed in anesthetized, male rats, 180–450 g, that were either normotensive (control) or had been made hypertensive by one of the following procedures:

(a) Grollman-type hypertension. Figure-of-eight surgical silk ligatures were applied to the left kidneys of etherized rats of the Holtzman Sprague–Dawley-derived (HSD) strain in the manner described by Grollman (9). The contralateral kidneys were left untouched. Experiments with SQ 20881 were performed with these rats 17–37 days after operation, by which time repeated caudal plethysmographic readings indicated systolic blood pressures > 150 mm Hg.

(b) Goldblatt-type hypertension. The left renal arteries of etherized rats of the Carworth Farms Nelson strain (CFN) were partially constricted with clips (i.d. 0.22 mm) fashioned from silver ribbon in a modification of the technique of Goldblatt (10). The right renal arteries were left unclipped. In some of these animals, the right kidneys were removed ("1-kidney" Goldblatt model) during the same operation. These rats were used in experiments with SQ 20881 6–21 days after operation, again after plethysmographic evidence of hypertension.

(c) Hypertension induced by aortic ligation. Ligation of the aorta with surgical silk between the origins of the renal arteries (11) was performed in etherized rats of the HSD strain. The animals were used in experiments with SQ 20881 6–8 days after operation when they had become hypertensive, as indicated by plethysmographic readings.

(d) Hypertension induced by unclamping an acutely clamped renal pedicle. Plastic-tipped hemostatic forceps were applied to both renal pedicles of HSD rats after they had been anesthetized (see below). Six hours thereafter, the left renal pedicle was unclamped and iv infusion of test compound was begun as soon as the resultant hypertension had stabilized (about 10 min after unclamping) (12).

Anesthesia was induced by the administration of sodium pentobarbital, 40 mg/kg, ip, in Grollman-type hypertensive rats, in rats with ligated aortas or clamped renal pedicles, and in some of the "2-kidney" Goldblatt-type hypertensive rats. In other models (normotensive "control" rats, "1-kidney" Goldblatt-type hypertensive rats, and the remaining "2-kidney" Goldblatt-type hypertensive rats) the anesthetic employed

[1] The initial demonstration of use of this class of peptides in ameliorating renal hypertension in three different models was first described by E. M. Krieger *et al.* [Lancet i, 269 (1971)] for the pentapeptide $BPP_{5a}$.

TABLE I. Effect of SQ 20881 on Mean Arterial Blood Pressure in Anesthetized Rats.[a]

| Model | SQ 20881 dose (mg/kg/min iv for 10 min) | Anesthesia | N | Pretreatment BP mean ± SE (mm Hg) | Change in BP: Max. decrease mean ± SE (%) | Change in BP: Mean ½ recovery time (approx.) (min) |
|---|---|---|---|---|---|---|
| Normotensive | (saline) | U | 5 | 101.2 ± 4.7 | 1.6 ± 2.7 | — |
| | 2.5 | U | 4 | 106.8 ± 5.5 | 26.0 ± 4.5 | 101 |
| Grollman | 2.5 | P | 5 | 150.5 ± 6.4 | 14.0 ± 6.1 | 2 |
| Goldblatt 1-kidney | 0.3 | U | 5 | 133.8 ± 6.7 | 16.0 ± 3.4 | 35 |
| Goldblatt 2-kidney | (saline) | U | 5 | 138.2 ± 7.0 | 2.4 ± 1.9 | — |
| | 0.3 | U | 4 | 154.5 ± 18.7 | 37.3 ± 10.7 | 43 |
| | 2.5 | U | 4 | 130.3 ± 12.7 | 28.8 ± 5.1 | 127 |
| | 2.5 | P | 4 | 164.0 ± 13.6 | 51.8 ± 10.5 | 12 |
| Aortic ligated | (saline) | P | 1 | 202.5 | 5.0 | — |
| | 0.6 | P | 3 | 205.8 ± 10.4 | 76.7 ± 17.2 | 9 |
| | 2.5 | P | 3 | 187.5 ± 10.0 | 35.8 ± 7.3 | >18 |
| Renal pedicle clamped | (saline) | P | 2 | (162.5, 180.0) | (12.5, 17.5) | — |
| | 2.5 | P | 4 | 158.4 ± 17.7 | 38.4 ± 4.8 | >28 |

[a] U = Urethane, P = sodium pentobarbital, N = number of rats per group. For the group of only 2 animals individual values are shown in parentheses.

was urethane, 1.5 g/kg, im. Polyethylene cannulas were implanted into the trachea, a femoral artery, and a jugular vein. Atropine sulfate was injected iv, 1 mg/kg, and heparin, iv, 250 U/kg. A Statham P23Gb pressure transducer coupled to a Beckman dynograph was used to monitor arterial blood pressure.

SQ 20881, dissolved in physiological saline, was infused once intravenously 0.3, 0.6, or 2.5 mg/kg/min for 10 min (infusion volume = 0.05 ml/min). In normotensive and in some Goldblatt-type hypertensive rats (both "1-kidney" and "2-kidney"), *stat* iv doses of angiotensins I and II, 0.1–1 μg/kg, dissolved in saline, were administered before, during, and after the infusion of SQ 20881 to determine the presumptive inhibition of angiotensin-converting enzyme as described pre-

TABLE II. Effect of SQ 20881 on Angiotensin I-induced Pressor Responses in Urethane–Anesthetized Rats.

| Model | SQ 20881 dose (mg/kg/min iv for 10 min) | N[a] | Angiotensin I response: Max. inhibition mean ± SE (%) | Angiotensin I response: Mean ½ recovery time (approx.) (min) |
|---|---|---|---|---|
| Normotensive | (saline) | 5 | 1.2 ± 3.6 | — |
| | 2.5 | 4 | 56.5 ± 14.0 | 102 |
| Goldblatt 1-kidney | 0.3 | 5 | 84.5 ± 9.7 | 75 |
| Goldblatt 2-kidney | (saline) | 5 | 5.9 ± 2.9 | — |
| | 0.3 | 4 | 69.1 ± 14.6 | 60 |
| | 2.5 | 4 | 47.4 ± 6.8 | ≧125 |

[a] N = Number of rats per group.

viously (1).

SQ 20881 was synthesized by Ondetti *et al.* (13) and Asp[1],Ile[5]-angiotensins I and II were purchased from Schwarz/Mann.

*Results.* Tables I and II summarize the data from these experiments. In most groups, renal-hypertensive as well as normotensive rats, administration of the nonapeptide SQ 20881 by intravenous infusion resulted in decreases greater than 25% in mean arterial blood pressure. Exceptions were the Grollman and the Goldblatt "1-kidney" groups, in which animals the decreases in blood pressure were only about 15%. On the other hand, marked vasodepressor activity was evident after treatment with the nonapeptide in all rats rendered hypertensive by the Goldblatt "2-kidney" technique, by aortic ligation, or by unclamping a renal pedicle. Maximal decreases in arterial blood pressure occurred 4–30 min after start of the infusion of SQ 20881. Typical blood pressure tracings from experiments with aorta-ligated and renal pedicle-clamped rats are shown in Fig. 1. Insofar as tested, no evidence of a dose–response effect was obtained, indicating perhaps that the doses chosen for these studies had produced maximal responses (compare 0.3 vs 2.5 mg/kg/min infusions in Goldblatt "2-kidney" animals and 0.6 vs 2.5 mg/kg/min infusions in rats with ligation of the aorta).

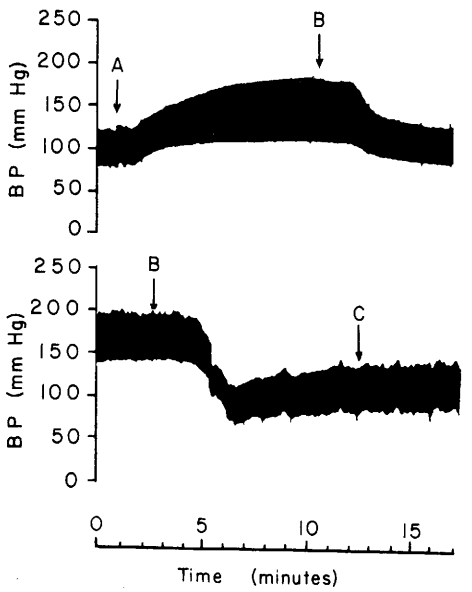


FIG. 1. Effect of SQ 20881 on mean arterial blood pressure (BP) in renal pedicle-clamped and aortic-ligated rats anesthetized with sodium pentobarbital. Upper tracing: Rat in which both renal pedicles had been clamped for 6 hr. At A, the left renal pedicle was unclamped. At B, an iv infusion of SQ 20881, 2.5 mg/kg/min, was started. Lower tracing: Rat in which the aorta had been ligated between the origins of the renal arteries 6 days previously. At B, an iv infusion of SQ 20881, 2.5 mg/kg/min, was started, and at C this infusion was stopped.

Presumptive evidence for the inhibition of angiotensin-converting enzyme was obtained in all SQ 20881-treated rats in which this activity was studied, *i.e.*, inhibition of vasopression evoked by *stat* iv injections of angiotensin I, but not of angiotensin II. The pressor activity produced by exogenous angiotensin II in these rats was unaltered or slightly augmented.

*Discussion.* The role of the renin–angiotensin system in the etiology of renal-induced hypertension continues to merit investigation. Different results are obtained in different models of experimental hypertension and in different animal species. Furthermore, the dosage levels of a hypotensive agent, as well as the specific anesthetic, if any, also influence the results.

It would seem likely that the vasodepressor effect produced by relatively large doses of SQ 20881 in anesthetized renal-hypertensive rats, as described here, resulted to some extent from an inhibition of angiotensin-converting enzyme in animals undergoing an abnormal increase in the formation of angiotensin II. In urethanized normotensive rats the nonapeptide also produced vasodepression and this occurred concomitantly with an inhibition of angiotensin I-induced pressor activity of about equal duration. This quantitative similarity in the response patterns of the two parameters studied suggests that the renin-angiotensin system was involved in the maintenance of the blood pressure of some, if not all, of these anesthetized test animals, and also that the vasodepressor activity of SQ 20881 was related, at least in part, to its known inhibitory effect on the angiotensin-converting enzyme.

No reduction in inhibitory effect of SQ 20881 on the pressor response to angiotensin I occurred, however, in unanesthetized genetically hypertensive rats dosed parenterally twice daily with $\leq$ 2 mg/kg/day for 5 days (3). Also, with further reference to the absence of anesthesia, inhibition of the pressor response to exogenous angiotensin I without decrease in blood pressure was observed in unanesthetized dogs receiving single parenteral doses of SQ 20881 $\leq$ 1 mg/kg (3). In dogs anesthetized with pentobarbital, similar effects were obtained with parenteral doses as small as 0.3 mg/kg (2). Intravenous infusion of SQ 20881 in a total dose of 0.5 mg/kg in acute renal pedicle-clamped rats treated with pentobarbital, phenoxybenzamine, and propranolol resulted in 30% inhibition of the pressor effect resulting from unclamping (14). In trained unanesthetized dogs, total parenteral doses of 1–5 mg of SQ 20881 inhibited the aortic pressor response in unilaterally nephrectomized dogs subjected to renal arterial constriction (15).

The relative activity of SQ 20881 in "1-kidney" Goldblatt rats underscores the different nature of the hypertension noted in this model, as compared with that in the "2-kidney" Goldblatt rat, a difference that has been reported by others (16, 17). The ineffectiveness of SQ 20881 in lowering the blood pressure of Grollman rats remains unexplained, although the comparatively long interval in this model between surgical manipulation to induce hypertension and experiments with SQ 20881 may have been a factor, since the amount of circulating renin would be expected to differ in the acute and chronic stages of hypertension.

In addition to inhibiting angiotensin-converting enzyme, SQ 20881 also augments the vasodepressor activity of bradykinin. It has been suggested that the kallikrein–kinin system may play a role in determining the level of blood pressure (18). It is not unreasonable to assume that the inhibitory activity of SQ 20881 on kininase may have contributed to the vasodepression seen in the present studies in rats anesthetized with urethane or pentobarbital. On the other hand, others (15) have reported little or no change in systemic renin activity, blood pressure, or concentration of plasma bradykinin after iv doses of 5–10 mg of SQ 20881 in unanesthetized dogs.

Determinations of blood levels of renin, angiotensin I, or kinins were not conducted in the tests on anesthetized rats reported herein; hence, it is difficult to ascribe a specific role to these factors in producing the hypotensive effects of relatively large doses of SQ 20881 (total doses 3–25 mg/kg). These doses of SQ 20881 were as much as ten to fifty times greater than those used previously (1–3, 14, 15) in either anesthetized or unanesthetized rats or dogs. Furthermore, other observations in this laboratory have indicated that even larger single doses of SQ 20881, 20–160 mg/kg, ip, had little or no effect on directly recorded blood pressure in genetically hypertensive rats.

SQ 20881 injected iv, 0.125–0.5 mg/kg, into normotensive men, had little or no consistent effect on blood pressure or on the pressor responses to injected angiotensin II, but decreased the pressor responses to exogenous angiotensin I (19).

*Summary.* 1. SQ 20881, the synthetic nonapeptide <Glu-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro, when infused iv at total dose levels of 3 to 25 mg/kg, lowered arterial blood pressure in normotensive rats and in three models of experimental renal hypertension, namely, "2-kidney" Goldblatt rats, aorta-ligated rats and "unclamped" renal pedicle-clamped rats, each anesthetized with urethane or pentobarbital. Such activity was questionable or not seen in "1-kidney" Goldblatt rats or Grollman rats.

2. SQ 20881 inhibited the pressor activity of exogenous angiotensin I, but not of angiotensin II, in normotensive rats and in "1-kidney" and "2-kidney" Goldblatt rats.

3. The pattern of the responses elicited by the administration of SQ 20881 suggests that when the peptide decreased blood pressure, it acted, at least in part, by inhibiting the activity of angiotensin-converting enzyme.

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