One prominent feature of the induction of mixed function oxidases by PCBs is that the enzyme activities, even after a single administration of the inducer, remain for weeks at elevated levels [5, 18]. The activities of epoxide hydrolase and glutathione S-transferase in rat liver, in a similar way, were above control levels still 4 weeks after a single i.p. injection of a commercial PCB mixture [5]. Compared with this, the decline of the enhanced enzyme activities was more rapid in the present study with mice.

To summarize, hepatic epoxide hydrolase and glutathione S-transferase activities of C57BL mice were increased after the i.p. injection of polychlorinated biphenyls (PCBs). The activities reached maximal levels 1 week after a single dose. The rise in epoxide hydrolase activity was generally higher than that of glutathione S-transferase. Enhancement of the activities of epoxide-metabolizing enzymes was most prominent in mice treated with hexachlorobiphenyls. The ability of PCB isomers to enhance the enzyme activities was different for various isomers, but no general rules of structure–activity relationship became apparent.

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Comparative study of three parenteral inhibitors of the angiotensin converting enzyme*

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Over the past few years, inhibition of the angiotensin converting enzyme (ACE, known also as kininase II) has been used for investigational purposes in animal experiments as well as for diagnosis and treatment of human hypertension. At present, two compounds are available for clinical use: teprotide (SQ20881; also known as BPP_{9a}), a nonapeptide suitable for i.v. administration only, and captopril (SQ14225), a modified proline molecule suitable for oral administration. Teprotide can be used for the diagnosis and initial treatment of selected hypertensive emergencies [1]. It has no known side-effects but is expensive and its supplies are limited. Captopril has been used successfully for long-term therapy of hypertension [2, 3] and congestive cardiac failure [4, 5], but it has been associated with a number of adverse reactions that appear to be more frequent and severe in patients with compromised renal function. Therefore, the search continues for new compounds capable of inhibiting the ACE.

Two such compounds have been investigated in the pres-

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Table 1. Magnitude and duration of the antihypertensive effects of the three inhibitors*

Drugs, in order of injection	BP (mm Hg)		Total duration of effect (min)	Half-recovery time (min)
	Before injection	After injection		
Group I (N = 7)				
(1) Teprotide (0.1 mg/kg)	207 ± 11	191 ± 10†	18.5 ± 5.0	11.0 ± 4.8
(2) GF 109 (0.1 mg/kg)	209 ± 11	190 ± 10†	19.7 ± 5.0	10.7 ± 2.0
(3) Teprotide (1 mg/kg)	211 ± 12	187 ± 10†	50.6 ± 9.0‡	24.8 ± 3.9
(4) GF 109 (1 mg/kg)	208 ± 11	184 ± 8†	53.0 ± 10.0‡	33.5 ± 6.6
Group II (N = 7)				
(1) GF 109 (0.1 mg/kg)	190 ± 10	177 ± 9†	18.8 ± 3.5	8.5 ± 1
(2) Teprotide (0.1 mg/kg)	191 ± 10	180 ± 9†	14.4 ± 2.7	5.4 ± 0.9
(3) GF 109 (1 mg/kg)	184 ± 16	164 ± 11†	79.0 ± 10.0‡	47.2 ± 15.0
(4) Teprotide (1 mg/kg)	182 ± 16	170 ± 11†	57.5 ± 6.0‡	24.0 ± 5.5
Group III (N = 6)				
(1) Teprotide (0.1 mg/kg)	198 ± 8	179 ± 8†	35.0 ± 6.0	24.6 ± 6.2
(2) GF 94 (0.1 mg/kg)	197 ± 7	176 ± 7†	52.8 ± 9.0	33.0 ± 13.0
(3) Teprotide (1 mg/kg)	200 ± 10	174 ± 8†	52.5 ± 8.5‡	27.0 ± 3.4
(4) GF 94 (1 mg/kg)	197 ± 12	170 ± 12†	67.0 ± 9.5§	23.5 ± 7.2
Group IV (N = 6)				
(1) GF 94 (0.1 mg/kg)	207 ± 5	184 ± 4†	27.8 ± 8.0	20.0 ± 9.5
(2) Teprotide (0.1 mg/kg)	198 ± 6	183 ± 7†	25.0 ± 8.8	16.1 ± 7.7
(3) GF 94 (1 mg/kg)	207 ± 8	181 ± 10†	62.0 ± 12.0‡	25.0 ± 6.0
(4) Teprotide (1 mg/kg)	198 ± 11	178 ± 10†	56.0 ± 12.0‡	19.5 ± 4.0

* Values for (1) blood pressures before injection and at the time of the maximum effect, (2) the duration of the effect of the drug, and (3) the time to one-half recovery for the three converting enzyme inhibitors at low and high doses in the four experimental groups.

† Different from baseline blood pressure, $P < 0.001$.

‡ Different duration from that of low dose, $P < 0.001$.

§ Different duration from that of low dose, $P < 0.01$.

ent study. The compounds are analogs of teprotide in which one or more of the proline residues has been replaced by L-3,4-dehydroproline. The analogs, hereafter called GF 94 and GF 109, are ΔPro^{9}-BPP_{9a} and $\Delta Pro^{3,9}$-BPP_{9a}. For reference, the primary structure of BPP_{9a} is Glu–Trp–Pro–Arg–Pro–Gln–Ile–Pro–Pro. GF 94 and GF 109 were found previously to be 125 and 50 times, respectively, more potent than teprotide in inhibiting the activity of the ACE *in vitro* [6]. Should such enhanced potencies be evident in *in vivo* studies, it might become economically feasible to use one or both analogs therapeutically [6]. Since teprotide has been shown repeatedly to reverse blood pressure elevation induced experimentally by renal artery stenosis [7], it is used as a standard to compare the antihypertensive effectiveness of the two new compounds in this same model. Our preliminary results indicate that both compounds are at least as potent and effective antihypertensive agents as teprotide.

Male Wistar rats, weighing 140–160 g, had a silver clip placed on the left renal artery with the contralateral kidney left intact (two-kidney, one-clip model of renovascular hypertension). They were maintained on Purina rat chow and tap water *ad lib.* for 4 weeks, by which time the animals had developed hypertension known to be angiotensin dependent [8]. On the day of the experiment the animal was anesthetized with ether. The external iliac artery was cannulated with a PE 50 catheter for blood pressure (BP) monitoring, and the femoral vein was cannulated with a PE 10 catheter for the infusion. Arterial pressure was monitored with a Statham Transducer and was recorded on a Hewlett–Packard recorder (model 7702B). After the animal recovered from anesthesia, it was kept in a semi-restrained position under a light mesh screen for 60–90 min, until blood pressure rose gradually to a steady baseline. At that point the first dose of the appropriate ACE inhibitor was injected as an i.v. bolus. Blood pressure was allowed to return to baseline after each injection, before the next one was administered. Each inhibitor was administered as a low dose (0.1 mg/kg body weight) and a high dose (1 mg/kg body weight). Each one of the two new inhibitors was compared against teprotide in each of the two doses. Thus, each animal received alternating injections of teprotide and one of the inhibitors at the low dose and subsequently at the high dose, for a total of four injections. Two groups received teprotide first, followed by one of the new inhibitors, whereas the other two received the new compound first followed by teprotide, to avoid misjudgment of the effect of the agent due to a possible lingering effect of the previous injection, despite the return of BP to baseline levels (see Table 1). Both the degree and the duration of the blood pressure fall induced by each inhibitor at each dose were compared with those obtained by teprotide. Results were analyzed by Student's *t*-test for paired or non-paired data as appropriate and presented as means ± S.E.M. Differences were considered to be significant at the $P < 0.05$ level.

ΔPro^{3}-BPP_{9a} (GF 94) and $\Delta Pro^{3,9}$-BPP_{9a} (GF 109) were synthesized as described previously [6]. The two analogs were relatively hydrated: GF 94, 475 nmoles/mg; GF 109, 710 nmoles/mg; expected, 908 nmoles/mg dry weight.

The aggregate effects of teprotide and of the two inhibitors on the blood pressure are shown in Fig. 1 for all experiments. Both the low and the high dose of each agent decreased mean blood pressure significantly from the baseline ($P < 0.001$). There was no significant difference between the blood pressure lowering effects of the two

doses for any of the compounds; however, the higher dose prolonged significantly the duration of this effect, which was 21 ± 3.5 versus 54 ± 4.5 min for the low and high doses of teprotide, respectively ($P < 0.001$), 19 ± 2.6 versus 63.5 ± 8 min for GF 109 ($P < 0.001$) and 36 ± 9 versus 64.5 ± 7.7 min for GF 94 ($P < 0.01$). The duration of the effect of each dose was the same for the three compounds. The lengths of time necessary for the blood pressure to return to the mid-point between baseline and maximum fall (time of one-half recovery) were similar after the injections of the experimental compounds and of teprotide at the same dose level. Table 1 presents in detail the blood pressure before, and at the time of, the maximum fall after injection for each group, as well as the total duration of, and time of one-half recovery from, the antihypertensive effect of each injection.

Figure 2 illustrates the maximum effect of each dose of new inhibitor on each animal plotted against the effect of the same dose of teprotide on the same animal. It is apparent that, regardless of whether the administration of the compound preceded or followed that of teprotide, the blood pressure obtained after its injection was almost identical to that of teprotide in most cases. The maximum antihypertensive effect was observed within 2–3 min after the injection of either dose of any one of the three compounds, regardless of order of administration.

We compared the antihypertensive efficacy of two polypeptide inhibitors of the angiotensin converting enzyme with that of teprotide, in terms of potency and duration of blood pressure lowering obtained at two different dose levels after intravenous administration.

Although GF 94 and GF 109 have been shown to be remarkably more potent than teprotide (BPP_{9a}) *in vitro* (125 and 50 times more potent respectively) [6], these two analogs do not differ clearly from teprotide *in vivo*. Tested on unanesthetized rats rendered hypertensive by renal artery constriction (two-kidney, one-clip model), all three compounds were equally effective for similar periods of time. In terms of doses expressed in mg/kg body weight, there is little reason to choose between teprotide, GF 94, and GF 109. Corrected for hydration, GF 94 (475 nmoles/mg dry weight; expected 908 nmoles/kg) may be twice as potent as teprotide. A doubling of potency, however, does not suffice to make GF 94 an economic alternative to teprotide nor to the cheaper, orally effective ACE inhibitor, captopril. Indeed, ΔPro, the uncommon amino acid, is not widely available and adds to the expense of preparing the analogs.

Nonetheless, there is ample reason to continue the search for alternatives to teprotide and captopril—the former because of expense and lack of oral effectiveness, and the latter because of adverse effects. For diagnostic purposes [1] and for treatment of emergencies such as congestive heart failure and malignant hypertension, oral effectiveness of an ACE inhibitor is not an absolute requirement. Despite its rapid onset of action after ingestion in the fasting state, the potency of the orally administered captopril is clearly reduced when food remains in the stomach. Because of its free mercapto-function and the consequent tendency to dimerization, however, captopril is not readily formulated into an injectable.

The use of an oligopeptide composed of common amino acids to inhibit the ACE for therapeutic purposes has great appeal. If the compound is degraded by the ACE or other enzymes, the component amino acids would be expected to enter physiologic stores or metabolic pathways. With current technology, however, there are clear limits to the size of an oligopeptide that can be made at costs reasonable to the patient. The expense of synthesis is a function of (among other things) the availability and costs of constituent amino acids, the efficiency of formation of peptide bonds, and the number of peptide bonds to be made. These considerations suggest that an inexpensive ACE inhibitor for use in medical emergencies probably will not be larger than a tri- or tetrapeptide. Whether a peptide of this size can be made to have high affinity for the ACE remains to be determined.

In summary, two new nonapeptide inhibitors of the agiotensin converting enzyme were compared with teprotide in terms of antihypertensive efficacy. When tested on unanesthetized rats with renovascular hypertension, the two new agents were similar to teprotide in potency, duration of action, and time of one-half recovery. Indeed, all three compounds induced pressure decrements in the order of 12–27 mm Hg within 2–3 min. The magnitude of the decrement was independent of the dose or order of administration, but higher doses prolonged the duration and time of half-recovery, which were similar for each dose of all three compounds. Thus, the two new agents may be useful as alternatives for the diagnostic and therapeutic indications of teprotide.

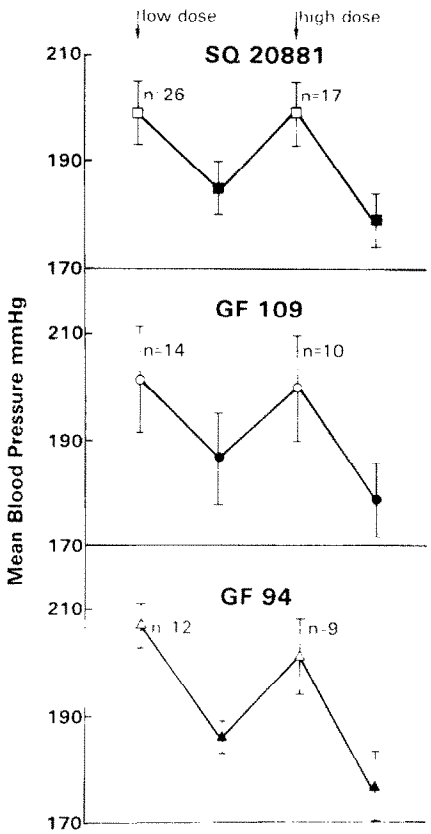

Fig. 1. Effects of the three angiotensin-converting enzyme inhibitors—teprotide, GF 109 and GF 94—on blood pressure of rats with renovascular hypertension. All animals which received the lower dose (0.1 mg/kg weight) or the higher dose (1 mg/kg weight) were grouped together regardless of the order of administration of each compound. There was no significant difference between the magnitudes of the antihypertensive effects caused by the two doses for the different compounds, but the higher dose produced significantly longer-lasting effects.

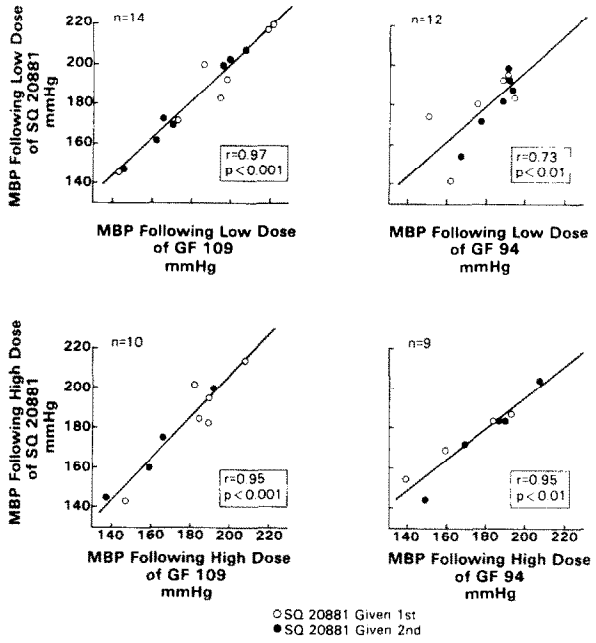

Fig. 2. Correlation of the antihypertensive effect of teprotide and the effect of each of the two new compounds at the same dose in the same animal. Key: (○) animals receiving teprotide first, followed by the new inhibitor; and (●) animals receiving first the new inhibitor, followed by teprotide.

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