We previously reported that hypotensive substances could be obtained from various plants through hot water extraction (Okamoto, et al., Jap Heart J 20:793, 1979). During screening tests, the extract from bamboo leaves, in particular, markedly decreased SHR and SHRSP blood pressures. Therefore, we tried to fractionate the hypotensive substances in bamboo leaves. The results of purification of and a pharmacological study on the effective substances are described in this report.

Materials and Methods A bamboo leaf extract was prepared by boiling leaves in 20 volumes of water for 1 hour. Treatment was the same as described in the above mentioned reference. The extract was dialyzed against water and the inner solution was salting out with (NH₄)₂SO₄ at 30% saturation. The precipitate was collected by centrifugation, dissolved in distilled water and dialyzed. The active precipitate thus obtained was fractionated on a column of Sephadex G-50 superfine. The 280 nm absorbance of the eluate was continuously monitored. Effluents were collected in each 20 ml fraction and appropriate aliquots analyzed for protein and sugar. Protein content was depended on the Lowry-Folin method (absorbance at 720 nm) and sugar content was measured by the Phenol-H₂SO₄ method (absorbance at 490 nm). The effective fraction was collected and then further fractionated by density-gradient isoelectric focusing. This fractionated substance was administered intravenously to SHR, SHRSP (blood pressures of 170 to 230 mmHg) and WKY. Blood pressure were measured chronologically using the tail-pulse pickup method without anesthesia. The effects of the fractionated substances on such contractive agents as K (2×10⁻³ g/ml), ACh (10⁻⁷ g/ml), NE (2×10⁻⁷ g/ml) and Ca (1 mM) were investigated the aortae of male WKY.

Results The hypotensive substances in bamboo leaf extract were fractionated by ultrafiltration, salting out and dialysis. When the salted out substance was subjected to ultrafiltration, a depressive effect was found for that portion with a molecular weight of under 30,000. This was fractionated on a column of Sephadex G-50 superfine and separated into four parts, A, B, C and D, according to the wave figure of the absorption at 280 nm. Fraction C, in comparison with the others, showed a remarkable hypertensive effect on blood pressures of SHR and SHRSP. Intravenous administration (3 mg/100 g body wt.) of fraction C induced a decrease in blood pressure of 128 mmHg 1 hour after injection; falls of 70 mmHg were observable even after 3 hours. The molecular weight of this fraction was under 10,000, and it had almost the same sugar and protein content as ratio. Fraction C was further separated by density-gradient isoelectric focusing into sub-fractions C-I, C-II and C-III. Both fraction C-I and C-II showed hypotensive effects but the later was more potent. The blood pressures of SHR and SHRSP fell by 43 mmHg 1 hour after injection (0.1 mg/100 g). The isoelectric point of C-II was located between 4 and 5, but this fraction did not give a single band on polyacrylamide gel (15%) at pH 8.9 as detected by Coomassie brilliant blue G-250. C-II may therefore be a phenolic substance. However, C-I gave a broad band, considered to be glycoprotein. Contractions by the above-named contractive agents were inhibited by this substance that is, ID₅₀ levels for K, ACh, NE and Ca were found to be 6×10⁻⁴, 5×10⁻⁴, 10⁻³ and 5×10⁻⁴ g/ml, respectively.

Summary The hypotensive substances in bamboo leaf extract were separated by dialysis, salting out, ultrafiltration and gel filtration. The most effective fraction was further fractionated by density-gradient isoelectric focusing. The isoelectric point of the fraction exhibiting a potent hypotensive effect on SHR and SHRSP was focused around a pH range from 4 to 5. On the basis of chemical analysis, this purified substance was considered to be either a glycoprotein or a phenolic substance with a molecular weight of 5,000 to 50,000. The hypotensive mechanisms may be ascribable to a direct effect upon the blood vessels.