Cardiovascular Effects of Mycelium Extract of *Ganoderma lucidum*: Inhibition of Sympathetic Outflow as a Mechanism of Its Hypotensive Action

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In an effort to understand the mechanism of cardiovascular actions of *Ganoderma lucidum* which was cultivated in Korea, the mycelium was isolated for a large-scale culture. Water extract of the mycelia was evaluated for its cardiovascular activity in anesthetized rabbits and rats. The left femoral artery and vein were cannulated for the measurement of arterial pressure and subsequent delivery of drugs. The left kidney was exposed retroperitoneally and a branch of the renal nerve was used to integrate renal efferent or afferent nerve activities. The extract decreased systolic and diastolic blood pressure, which was accompanied by an inhibition of renal efferent sympathetic nerve activity. The extract did not decrease heart rate in these animals, although there was clear hypotension in the extract dose dependent manner. This suggests that the hypotension induced by the treatment of the extract was secondary to the primary effect of the extract in the central nerve system, which suppressed the sympathetic outflow. Therefore we concluded that the mechanism of hypotensive action of *Ganoderma lucidum* was due to its central inhibition of sympathetic nerve activity.

Keywords *Ganoderma lucidum*; blood pressure; renal nerve activity; heart rate; sympathetic outflow; hypertension

Introduction

For centuries *Ganoderma lucidum* (Fr.) KARST has been used for a variety of medical problems such as rheumatism, asthma, chronic bronchitis, and even for the purpose of sedation.[2] Previous researches indicate that *Ganoderma lucidum* has effective components as antihypertensive,[3-6] antineoplastic,[7-11] hypolipidemic,[12] antiplatelet coagulation,[13] inhibition of histamine release from the mast cell,[14] and diabetes mellitus.[15] Many putatively effective compounds were identified and isolated from *Ganoderma*. For instance, lanostan type triterpens such as ganoderic acids, ganolecidic acids, ganoderans, lucidenic acids, and lucidones were known to be the main chemical entity of the family of *Ganoderma*.[16-22]

However, little is known about the pharmacological action of *Ganoderma*, even though it has been reported that the various forms of *Ganoderma* extract reduced blood pressure in spontaneous hypertensive rats[3,6] and in essential hypertensive human subjects.[4,5] The primary purposes of the present study were to: 1) examine the cardiovascular effects of *Ganoderma*, and 2) determine if *Ganoderma* decreases blood pressure as a direct result of its suppression of sympathetic outflow.

Materials and Methods

Myselium was separated from *Ganoderma lucidum* (Fr.) KARST, which was cultivated in Korea, and was cultured in a medium for two weeks according to the method of Kang *et al.*[8] The myselium mass was extracted as described in Fig. 1, of which extract will be referred as myselia extract of *Ganoderma* (MEG) hereafter.

Animal Preparations Male New Zealand rabbits (1.5—3.0 kg) were anesthetized with urethane (1 g/kg) intraperitoneally. The left femoral artery and vein were cannulated, using polyethylene tube 50 (Clay Adams, Parsippany, NJ) for the direct recording of blood pressure and administration of drug, respectively. The animals were allowed to respire spontaneously through a tracheostomy, and were maintained at 37 °C with a temperature regulator (Gorman Rupp, Bellville, OH) for the anesthetized rabbit preparation. For conscious rabbit preparations the animals were anesthetized with pentobarbital (30 mg/kg, i.v.) instead of urethane. The right carotid artery and the left jugular vein were cannulated with polyurethane tube (Microrenathane, 0.04 in, o.d., 0.025 in, i.d., Braintree Sci., MA). The catheters were exteriorized to the nape of the animals through a tunnel passage under the skin. Conscious Sprague Dawley rats (250—300 g) were also prepared similarly, using aseptic instruments under aseptical conditions as reported previously.[23] After a recovery from the surgical anesthesia the animals were subjected to drug test as described below.

Recording of Physiological Parameters and Renal Nerve Activity Arterial pressure was monitored by a Statham P23 pressure transducer coupled to a pressure processor amplifier (Gould Instruments, Cleveland, OH, model 13-4615-52) which computes the systolic, mean, diastolic, and pulse pressures. Heart rate was monitored by a Gould biotec electrocardiogram amplifier (model 13-4615-65), *via* subcutaneous platinum electrodes (lead II). Tracheal pressure was also estimated by a Gould pressure transducer (model PM 15E) similarly.

After the basic surgery, the animals were placed on their right side and the kidney was exposed retroperitoneally. A branch of the left renal nerve was carefully dissected from the surrounding connective tissue, cleaned, and suspended on a bipolar electrode in a pool of mineral oil, as reported previously.[24] The nerve was cut in half and renal efferent nerve activity (RENA) was recorded, using two electrodes which were placed on the proximal end of the nerve, while renal afferent nerve activity (RANA), was determined from the distal half of the nerve as described below. Multiunit, renal nerve activity (RNA) was monitored from intact whole nerve bundle by a Gould universal amplifier (model 13-4615-56) and was integrated with respect to time by a Gould integrator (model 13-4615-70) in 2 s intervals. All of the above parameters were recorded on a Gould 16-channel electrostatic recorder (model ES 1000) as reported previously.[24]

In order to test the validity of the nerve preparation, a reflex withdrawal of sympathetic discharge was produced by an i.v. injection of norepinephrine (4 μg/kg) or phenylephrine (20 μg/kg). Elimination of recorded activity after the rise in blood pressure verified the measurement of multiunit nerve activity and that ongoing activity was predominantly sympathetic.[24]

Administration of Drug and Statistical Analysis MEG was dissolved in saline at room temperature and centrifuged at the speed of 3000 rpm. The clear supernate was infused *via* the femoral or jugular vein catheter, using a syringe pump (Sage Inst., Cambridge, MA, model 341A) in a volume of 0.25 ml. The MEG doses were 3, 10, and 30 mg/kg and the same volume of saline was infused identical manner as a control. Approximately 15 min was allowed between MEG treatment or until the cardiovascular parameters returned to baseline values. The mean values for arterial pressure, heart rate, and renal nerve activity were expressed as function of the doses of MEG. Time for half-maximal recovery (T50) of systolic pressure after the maximal decrease of blood pressure was calculated in the control and drug-treated groups. The whole renal nerve activity as well as its efferent and afferent activities were expressed as a percentage of change after normalization of the values obtained with different doses of MEG. Overall slopes of the lines were analyzed by a two-way analysis of variance and each datum point was analyzed by unpaired Student *t*-test. Significant difference was considered as p is less than 0.05.

Results

Experiments with Anesthetized Rabbits Figure 2 shows

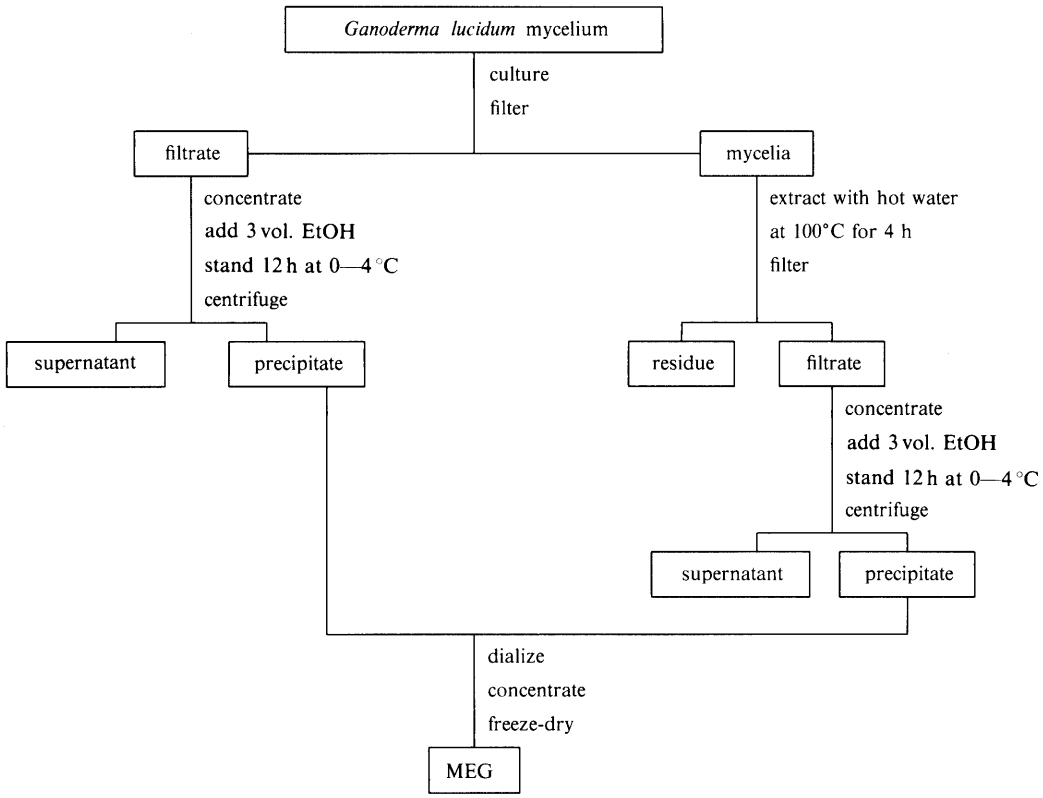

Fig. 1. Flow Chart for the Preparation of an Extract from *Ganoderma* Mycelia Isolated from *Ganoderma lucidum* (Fr.) KARST

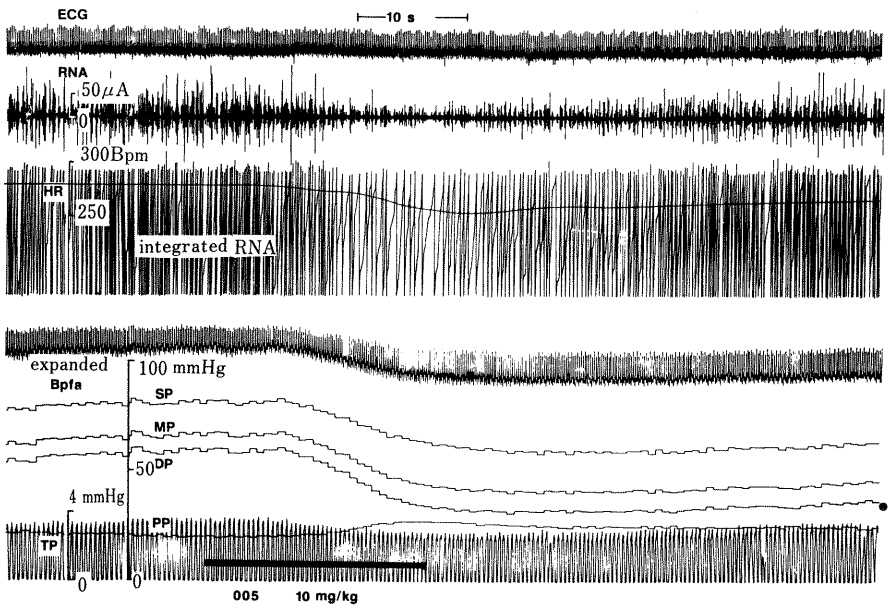

Fig. 2. A Typical Effect of Mycelia Extract of *Ganoderma* (MEG) on Electrocardiogram (ECG), Renal Nerve Activity (RNA), Heart Rate (HR), Femoral Arterial Blood Pressure (BPfa) and Tracheal Pressure (TP) in Anesthetized Rabbit

Bpm, sp, mp, dp and pp stand for beats per minute, systolic, mean, diastolic, and pulse pressure, respectively. MEG (10 mg/kg) was infused for the duration indicated by a line at the bottom.

typical effects of MEG (10 mg/kg) on electrocardiogram (ECG), RNA, heart rate (HR), femoral arterial blood pressure (BPfa) and tracheal pressure (TP), in anesthetized rabbits. MEG decreased systolic, mean, and diastolic pressure as well as heart rate, including the RNA. An inspection of the chart also shows that the suppression of the RNA came simultaneously with the reduction in blood pressure and heart rate, which can be quantitated in integrated RNA. There was a net increase in pulse pressure because the decrease in the diastolic pressure was greater than the reduction in systolic pressure.

Onset of these effects after the MEG administration was usually manifested in 10 to 20 s depending upon the dose used. The time for a peak MEG action, however, was

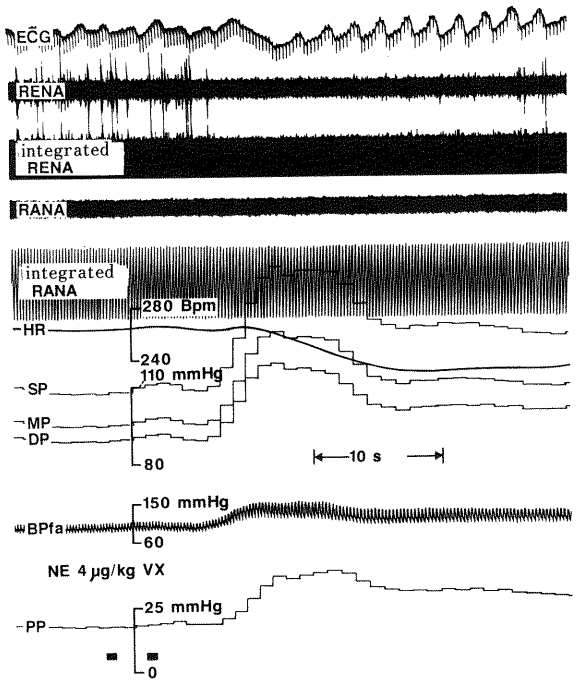

Fig. 3. Effects of Norepinephrine (NE) on Electrocardiogram (ECG), Renal Efferent Nerve Activity (RENA), Renal Afferent Nerve Activity (RANA), Heart Rate (HR), and Femoral Arterial Blood Pressure (BPfa) in Anesthetized Rabbit

Bpm, sp, mp, dp, and pp stand for beats per minute, systolic, mean, diastolic, and pulse pressure, respectively. NE was loaded at the first bar at the bottom and subsequently displaced by 0.25 ml of saline at the second bar.

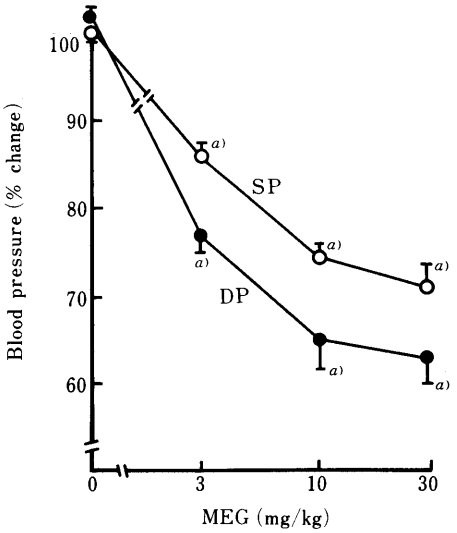

Fig. 4. The Dose-Response Relationship of MEG on Systolic and Diastolic Pressure in Anesthetized Rabbits

Animals were prepared as in Methods, and MEG was infused *via* the femoral vein. Each value represents mean of at least 5 experiments. Vertical bars indicate SEM and *a*) indicates p is less than 0.01.

independent from the dose of the drug, which fell on 25 to 50 s. The duration of MEG action was drug dose dependent. That is, in the case of 10 mg/kg, at least 2 min, was required to overcome the cardiodepressive action of MEG, although it appears that the recovery rate of the RENA was faster than the blood pressure. An injection of norepinephrine (4 μg/kg) produced hypertension with a rapid and virtually complete cessation of RENA in every animal used (Fig. 3). In each case, the blood pressure rise induced by

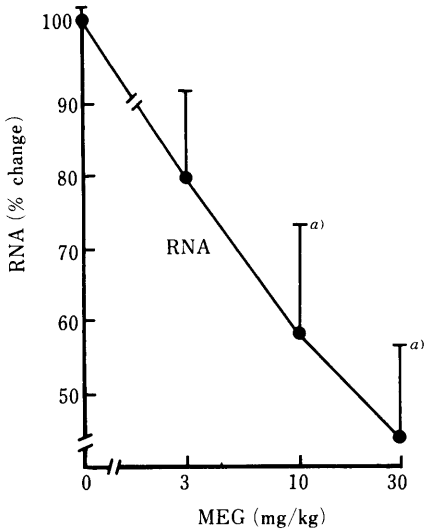

Fig. 5. Effect of MEG on the Inhibition of Renal Nerve Activity (RNA) in Anesthetized Rabbits

Signs and abbreviations were used as in Fig. 4. *a*) Indicates p is less than 0.05.

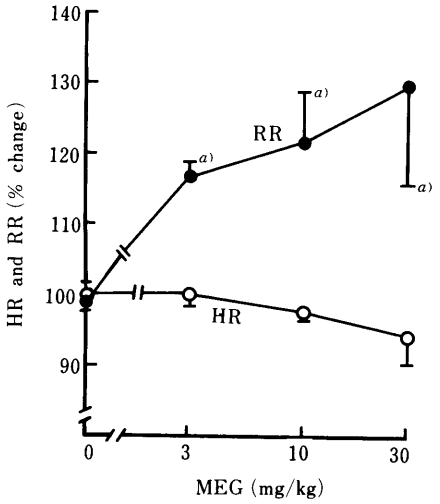

Fig. 6. Effects of MEG on Heart Rate and Respiration Rate in Anesthetized Rabbits

Abbreviation and signs were used as in Fig. 4. *a*) indicated p is less than 0.05.

norepinephrine occurred before the reduction of RENA and heart rate. In this Fig. 3, norepinephrine has no effect on RANA and its integrated RANA.

The dose dependent effect of MEG on percentage of decrease in arterial blood pressure is illustrated in Fig. 4. At the dose of MEG, 3 mg/kg, as much as 25% reduction in diastolic pressure was seen, though there was about 15% decrease in systolic pressure. In all doses of MEG tested in the present study there was at least 10% more reduction in diastolic pressure than the decrease in systolic pressure. Figure 5 shows the MEG dose dependent reduction of renal nerve activity in 5 anesthetized rabbits. MEG suppressed about 20% of RNA at 3 mg/kg while it effectively reduced the discharge for 40% at 10 mg/kg. MEG not only reduced the rate of RNA discharge, but also virtually stopped the activity for several seconds depending on its doses.

Effects of MEG on heart rate and the rate of respiration are summarized in Fig. 6. MEG had no statistically significant effect on heart rate, although a trend of its effect on

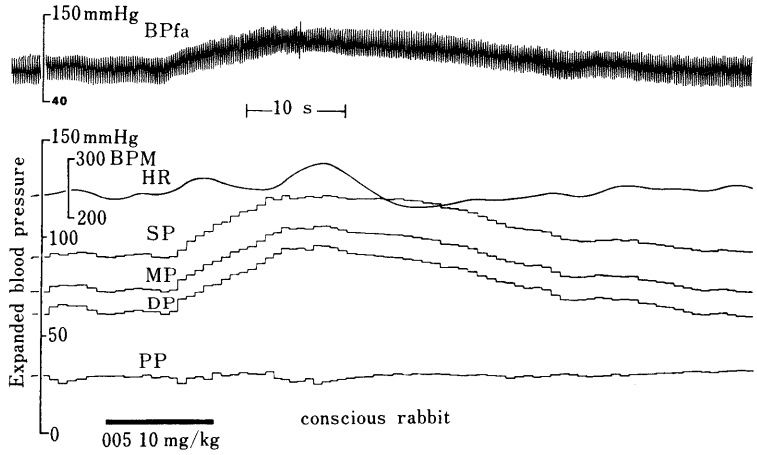

Fig. 7. A Typical Effects of MEG on Femoral Arterial Blood Pressure (BPfa) and Heart Rate (HR) in Conscious Rabbit

A conscious rabbit was prepared as described in Methods, and MEG was infused *via* the jugular vein for the duration indicated by the bar at the bottom. Bpm, sp, mp, dp, and pp stand for beats per minute, systolic, mean, diastolic, and pulse pressure, respectively.

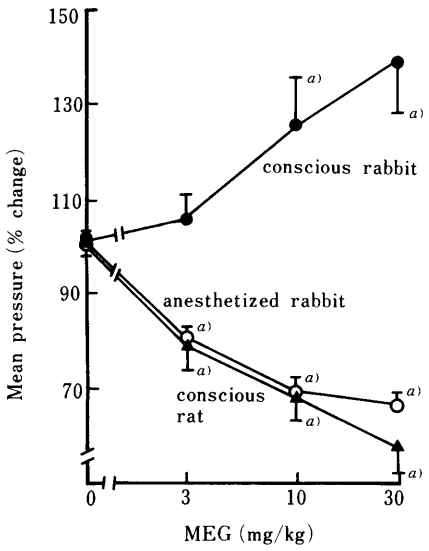

Fig. 8. Comparison of MEG Effect on Mean Pressure in Conscious Rabbits, Anesthetized Rabbits, and Conscious Rats

A total 10 rabbits were divided into two groups, five each, to prepare conscious and anesthetized preparations. Six rats were used, and all other signs were used as in Fig. 4.

heart rate was rather clear. In general, the rate of respiration was increased as the dose of MEG was increased as in Fig. 6. As much as 30% increase in respiration rate was evident after the dose of MEG, 30 mg/kg.

Experiments with Conscious Rabbits and Rats In an attempt to compare the effect of anesthesia on MEG action, the effects of MEG were also evaluated in conscious animals. Figure 7 shows a typical effect on BPfa, and HR in conscious rabbits. Contrast to the effect of MEG in anesthetized rabbits, it increased systolic, mean, and diastolic pressure, which was evident in 10 s after the onset of MEG infusion. The peak effect was also manifested quickly and, likewise, the duration of its action was short, compared to those in the anesthetized rabbits (Fig. 2). There were no significant changes in either heart rate or pulse pressure in the conscious rabbits.

Figure 8 summarizes mean pressure obtained from conscious rabbits and rats after an infusion of different doses of MEG. The mean pressure was increased as the dose of MEG increased in the conscious rabbits. However, in case of urethane anesthetized rabbits, MEG distinctly decreased the parameter in dose dependent manner as presented (Fig. 4). Interestingly, MEG decreased significantly arterial blood pressure in conscious rats (Fig. 8).

Discussion

Although it has been documented that effectiveness of *Ganoderma* is well known for many different pathological systems (see Introduction), different laboratories have produced quite variable results as to its circulatory effects such as on systemic blood pressure. Water extract of *Ganoderma* obtained from Kyoto and Otawara, Japan, reduced systolic pressure in conscious spontaneous hypertensive rats (SHR) and in conscious Wistar rats after an oral administration of the extract.[3] The antihypertensive effect was moderate such that there was about 10% decrease in the systolic pressure. The effective component was appeared to be present in a fraction which contained more than 100000 dalton, based on gel fractionation.

Water soluble extract of *Ganoderma* cultivated in Korea also exhibited its dose dependent antihypertensive action in pentobarbital anesthetized SHR.[6] The extract reduced systolic pressure by 30% and diastolic pressure by 40%, depending on the extracts of *Ganoderma* preparations. The extract simultaneously reduced heart rate approximately 30% in spite of the reduction of blood pressure.[6] They also studied the direct effect of *Ganoderma* on isolated blood perfused *in situ* canine heart, using another blood donor dog.[6] One of their *Ganoderma* preparations increased cardiac contractility, heart rate, and coronary blood flow while another preparation decreased heart rate without a pronounced effect on cardiac contractile force.

The main purpose of our study was to examine cardiovascular effects of mycelia extract of Korean *Ganoderma* (MEG), since in most of other studies, they used *Ganoderma* mushrooms. The MEG decreased blood pressure without baroreflex-mediated increase in heart rate in anesthetized white New Zealand rabbits and conscious Sprague-Dawley rats. In these animals, a simultaneous reduction in RENA was observed, which suggests indirectly the causal relationship between MEG induced hypotension and inhibition of RENA. That is, MEG somehow de-

creased the general sympathetic outflow so that RENA was blocked before the subsequent reduction in arterial blood pressure.

Additionally, this compels the idea that MEG acts primarily on the central nervous system (CNS) to curtail the peripheral sympathetic nerve activity, which dictates to decrease the arterial blood pressure. It may not be clear the temporal relationship between RNA and blood pressure from Fig. 2 since the rate of recording was rather slow. Whenever we increased the speed of Chart 5 to 10 times of the original rate, then it was not difficult to see the reduction of RNA before the subsequent decrease in blood pressure as reported previously.[24] Had MEG injection acted peripherally to lower blood pressure, a baroreflex increase in RENA and tachycardia would have been anticipated. This is internally consistent with the fact that MEG did not increase heart rate (Fig. 2). On the contrary, it decreased heart rate at the high dose as shown in Fig. 6. The onset of the MEG hypotensive action was fast (10 to 20 s), which might raise a question as to the permeability of effective component(s) of MEG into the blood brain barrier. Since the putative active entity of MEG has not been identified, small molecules or their metabolites of MEG, *in vivo*, might be the one which is responsible for MEG action in the CNS.

The results of our experiments with conscious rats and anesthetized rabbits are consistent with the findings reported previously,[3,6] despite the fact that we used an extract of a cultured *Ganoderma* mycelia instead of *Ganoderma* mushroom as a whole. However, it is extremely difficult to make generalization as to the actions of this class drug due to the fact that different investigators used different variety of *Ganoderma*, location of cultivation, methods of isolation and extraction, different degree of purity, and animal species they used in different experimental conditions. For instance, in conscious rabbits, MEG increased blood pressure transiently without a significant change in heart rate in this present study (Fig. 7 and 8). Many anesthetic agents eliminate cortical influence and other central inputs from the brain stem, particularly ventrolateral medulla (C_1 neurons), which dictates sympathetic discharge and subsequent blood pressure. Indeed, there was a good temporal correlation between the two parameters in urethane anesthetized rabbits. In conscious animals MEG responses were, in general, variable compared to those in anesthetized animals, due to presumable compensatory reflex. Methionine enkephalin is known to decrease blood pressure in anesthetized animals, while it may increase the parameter in conscious animals.[24-26]

In human studies, Arichi *et al.*[4] reported that *Ganoderma* extract decreased blood pressure in essential hypertensive individuals with or without simultaneous medication of other antihypertensive agents. They also noted that their *Ganoderma* preparation decreased serum total cholesterol and triglyceride. Interestingly, this antihypertensive effect of *Ganoderma* was only manifested in essential hypertensive patients in another study,[5] since the *Ganoderma* preparation had little or no effect on normotensive as well as mild hypertensive human subjects. There was no apparent adverse effect of this drug on biochemical or hematological variables even after oral administration of this preparation for six months. In this present study we did not test the potential antihypertensive action of MEG since we used normotensive rats without any other medications. It would be fruitful to investigate the cardiovascular action of MEG in spontaneous hypertensive rats with a simultaneous determination of sympathetic efferent nerve activity. Although our main research interest was to define the fundamental mechanism(s) of MEG cardiovascular actions, differential pharmacological action of this compound, depending on its origin and purity, suggests effective compounds must be individually isolated, characterized and purified in a homogeneity before meaningful pharmacological test.

In the present study, MEG reduced sympathetic nerve discharge without an increase in afferent nerve activity in the same nerve preparation. There was no increase in heart rate, although there was a significant decrease in blood pressure after different doses of MEG. The reduction of sympathetic discharge was occurred before the reduction in blood pressure in temporally concerted manner. The electrophysiological evidence strongly supports the concept of central site(s) of MEG action. However, this does not preclude the potential peripheral site(s) of MEG actions. MEG or its metabolites might act sympathetic ganglia, which will reduce the release of norepinephrine, although this is highly unlikely since our electrodes monitored sympathetic nerve activity at postganglionic sympathetic fiber. Our technique could not test the direct action of MEG on smooth muscle or adrenergic receptors. If it acted on blood vessels directly, the resulting hypotension would increase dramatically afferent nerve activity, which we failed to observe in this experiment. In our preliminary studies, MEG also increased cardiac contractile force and the first derivates of the left ventricular pressure in isolated perfused rabbit heart, instead of a reduction of cardiovascular function.

In conclusion, this study confirmed that MEG had definite effect on cardiovascular system such as reduction in blood pressure, renal nerve activity, and heart rate in anesthetized as well as conscious animals. The hypotensive action of MEG was always manifested with the reduction of sympathetic outflow, which explains the mechanism and the locus of the hypotensive action of MEG. Needless to say, further pharmacological study is necessary, particularly for the elucidation of the exact mechanism and locus of MEG action in the central nervous system, using a single chemical entity of MEG extract.

References and Notes

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