Title

EFFECT OF AMLODIPINE, A CALCIUM CHANNEL ANTAGONIST, ON CHOLESTEROL LEVELS IN THE CEREBRAL CORTEX AND HIPPOCAMPUS OF OBESE AND HYPERTENSIVE SHR.Cg-Leprcp/NDmcr RATS

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EFFECT OF AMLODIPINE, A CALCIUM CHANNEL ANTAGONIST, ON CHOLESTEROL LEVELS IN THE CEREBRAL CORTEX AND HIPPOCAMPUS OF OBESE AND HYPERTENSIVE SHR.Cg-*Lepr*^{cp}/NDmcr RATS

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SUMMARY

1. It has been suggested that hypertension, hyperlipidemia and diabetes participate in the onset and development of dementia. We estimated the effect of the antihypertensive drug amlodipine, a calcium channel antagonist, on blood pressure, plasma lipids and cholesterol levels in the detergent insoluble membrane fractions (DIFs) of the cerebral cortex and hippocampus in obese and hypertensive SHR.Cg-*Lepr*^{cp}/NDmcr (SHR-cp) rats.

2. Twelve male SHR-cp rats (10 weeks old) were randomly divided into two groups: a vehicle group and an amlodipine group administered amlodipine (8 mg/kg per day) orally for 9 weeks.

3. Bodyweight did not differ between the two groups, but the level of systolic blood pressure in the amlodipine-administered SHR-cp rats was significantly lower than in the vehicle rats.

4. The cholesterol levels of plasma and the DIFs in the cerebral cortex and hippocampus were both significantly higher in the amlodipine-administered SHR-cp rats than in the vehicle rats. Regression analysis revealed a significant positive correlation between plasma cholesterol and the cholesterol level in the DIFs of the hippocampus.

5. These results suggest that the long-term administration of amlodipine may induce antihypertensive and hypercholesterolemic effects in obese and hypertensive SHR-cp rats.

Key words: antihypertensive drugs, brain cholesterol, hypercholesterolemia, lifestyle-related diseases, model rats, neural cell membrane.

INTRODUCTION

The association between cognitive dysfunction and nutrition in the elderly is currently drawing attention. The prevailing wisdom is that high cholesterol is a risk factor for dementia and recent epidemiological studies suggest that individuals taking cholesterol-lowering drugs are at a reduced risk of Alzheimer's disease (AD).[1] We reported that the impairment of cognition learning ability is associated with the increased cholesterol levels in the detergent insoluble membrane fractions (DIFs) of the cerebral cortex and hippocampus of rats infused amyloid β peptide into the cerebral ventricle.[2] The SHR.Cg-*Lepr*^{cp}/NDmcr (SHR-cp) rat, one of the metabolic syndrome model rats, is a new, genetically obese strain that develops obesity, hypertension, hyperlipidemia and hyperinsulinaemia, the so-called lifestyle-related diseases.[3] Few studies have been conducted, however, on the pharmacological effects on this rat. Since amlodipine (a calcium channel antagonist), one of the typical antihypertensive drugs, shows hypocholesterolemic effects in normal and hypercholesterolemic rats[4] and in hypertensive patients,[5] estimating the effects of the drug on SHR-cp rats is significant. In this study, we estimated the effect of amlodipine on blood pressure, plasma lipids and the cholesterol levels in DIFs of the cerebral cortex and hippocampus of SHR-cp rats.

METHODS

Twelve male SHR-cp rats (10 weeks old; Japan SLC, Hamamatsu, Japan), housed in a room under controlled temperature (23 ± 2°C), relative humidity (50 ± 10%) and light–dark cycles (light, 8:00–20:00; dark, 20:00–8:00), were fed laboratory chow (labo MR Stock; NOSAN Corporation, Yokohama, Japan) and given water *ad libitum*. The rats were cared for and killed in accordance with the Guidelines for Animal Experimentation of the Japanese Association for Laboratory Animal Science. The rats were randomly divided into two groups: a vehicle group (Con rats) and an amlodipine group (8 mg/kg per day, Amlo rats) administered amlodipine orally for 9 weeks. Before and after amlodipine administration for 9 weeks, the rats were weighed and systolic blood pressure was measured by the tail-cuff plethysmographic method (UR-1000; Ueda, Tokyo, Japan). After an 18-hour overnight fasting period, the rats were then anaesthetized with sodium pentobarbital (65 mg/kg bodyweight, i.p.), their blood collected and the cerebral cortex and hippocampus separated on ice.

Plasma levels of triglyceride and total cholesterol were measured by an enzymatic colour test using commercial kits (the Total Cholesterol *E*-test and the Triglyceride *E*-test; Wako Pure Chemical, Osaka, Japan).

DIFs in the cerebral cortex and hippocampus were prepared and the cholesterol level in DIFs was measured by gas chromatography, as previously described.[2]

Results are expressed as the means ± SEM. Data were evaluated by regression analysis and by paired and unpaired Student's *t*-tests, using the computer program StatView 4.01 (MindVision Software; Abacus Concepts, Berkeley, CA, USA). A level of $P < 0.05$ was considered statistically significant.

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Table 1 Physical and plasma characteristics and the cortico-hippocampal levels of cholesterol in the detergent insoluble membrane fractions (DIFs) in vehicle- and amlodipine-administered SHR.Cg-*Lepr*^{cp}/NDmcr rats

	Vehicle ($n = 6$)		Amlodipine ($n = 6$)	
	Before	After	Before	After
Bodyweight (g)	279 ± 15	547* ± 14	280 ± 10	539* ± 9
Heart rate (b.p.m.)	379 ± 10	401 ± 8	397 ± 15	396 ± 14
Systolic blood pressure (mmHg)	128 ± 6	188* ± 2	130 ± 9	164*,** ± 6
Plasma total cholesterol (mg/dL)	170 ± 11	168 ± 12	181 ± 6	216*,** ± 13
Plasma triglyceride (mg/dL)	393 ± 36	466 ± 39	386 ± 81	549* ± 48
Total cholesterol in DIFs (mg/mg DIFs protein)				
Cerebral cortex	ND	1.31 ± 0.07	ND	1.54** ± 0.06
Hippocampus	ND	2.22 ± 0.19	ND	2.83** ± 0.18

Data are the mean ± SEM. *$P < 0.05$ compared with before. **$P < 0.05$ for amlodipine compared with vehicle. DIFs, detergent-insoluble membrane fractions; ND, not determined.

RESULTS

Physical and plasma characteristics and the cholesterol levels in the DIFs in the cerebral cortex and hippocampus in Con and Amlo rats are shown in Table 1. Bodyweight did not differ between the two groups. The level of systolic blood pressure in the Amlo rats was significantly lower than in the Con rats, indicating the anti-hypertensive effect of the drug.

Plasma levels of cholesterol were significantly higher in Amlo rats than in Con rats, indicating the hypercholesterolemic effect of the drug. The plasma triglyceride level in SHR-cp rats was not affected by the drug.

In Amlo rats, the levels of cholesterol increased in the DIFs of the cerebral cortex and hippocampus compared with those in the vehicle rats. Regression analysis revealed a significant positive correlation between plasma cholesterol and the cholesterol level in the DIFs of the hippocampus ($r = 0.662$, $P = 0.0190$).

DISCUSSION

Amlodipine reduces plasma cholesterol levels in normal and hypercholesterolemic rats[4] and in hypertensive patients.[5] In this study, however, the administration of amlodipine increased the plasma cholesterol level in SHR-cp rats, a contradiction difficult to explain at this time. An increase in the dietary intake of Ca dose-dependently decreases serum total cholesterol in obese Zuker rats, because of a Ca-induced increase in the conversion of cholesterol into bile acids.[6] Activation of the Ca channel may, thus, participate in the conversion, leading to a decrease of plasma cholesterol in the rats. These results suggest that amlodipine administered to SHR-cp rats may increase plasma cholesterol, presumably attributed to its calcium channel-blocking effect.

We have demonstrated that the impairment of spatial cognition learning ability induced by the infusion of amyloid β peptide into the rat cerebral ventricle is associated with an increase in the cortico-hippocampal levels of cholesterol in DIFs.[2] In the present study, amlodipine increased the cholesterol content in the DIFs of the hippocampus concurrently with a significant increase of plasma cholesterol in SHR-cp rats. To date, there are no reports on the hypercholesterolemic effects of amlodipine. Although the enhancing effect of amlodipine on the cholesterol content of plasma and on hippocampal DIFs may be a finding specific to obese and hypertensive SHR-cp rats, the present study suggests that the long-term administration of amlodipine may affect the cholesterol metabolism and the physiological function of the brain in the SHR-cp rats with lifestyle-related diseases. Further investigations are needed to clarify the hypercholesterolemic effect of amlodipine in SHR-cp rats.

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