MOLECULAR PHARMACOLOGY, 17, 137-140

SHORT COMMUNICATION

Abnormal Biochemistry of Vascular Smooth Muscle Plasma Membrane Isolated from Hypertensive Rats

C.-Y. KWAN, L. BELBECK AND E. E. DANIEL

Department of Neurosciences, McMaster University, Hamilton, Ontario, L8S 4J9 Canada

Received July 30, 1979; Accepted September 11, 1979

SUMMARY

KWAN, C.-Y., L. BELBECK AND E. E. DANIEL. Abnormal biochemistry of vascular smooth muscle plasma membrane isolated from hypertensive rats. *Mol. Pharmacol.* 17: 137-140 (1980).

We have demonstrated that the mesenteric arteries of rats with either genetic hypertension or experimental hypertension induced by dexoycorticosterone have similar biochemical alterations which were manifested by (a) increased amount of total wet weight of arteries as well as total protein content of isolated arterial plasma membranes, (b) increased alkaline phosphatase activities in the plasma membranes of arterial smooth muscle, and (c) decreased ATP-dependent transport of calcium by the arterial plasma membranes. The observed abnormal biochemical properties cannot be attributed to the use of different strains of normotensive control rats in the case of genetic hypertension. Deoxycorticosterone treatment alone slightly enhanced alkaline phosphatase activity but did not alter calcium accumulation, weight or protein content of plasma membranes from arterial smooth muscle. The results suggest that abnormal biochemistry of the plasma membrane isolated from small arteries of hypertensive animals, which appears to involve a defect of calcium regulation across the vascular plasma membrane, is probably associated with the pathogenesis of hypertension.

INTRODUCTION

Studies from several laboratories have shown evidence that derangement of cellular Ca^{2+} regulation in vascular smooth muscle occurred in rats with established hypertension (1-4). Our recent study has also shown that the altered Ca^{2+} regulation, as well as several enzymatic activities, was observed primarily in the plasma membrane-enriched fraction of the aorta from rats with genetic hypertension (5). Little information is available about small blood vessels in hypertensive animals, even though small resistance vessels play the major role in determining peripheral vascular resistance. We have previously reported a procedure for the isolation of subcellular membrane fractions from rat mesenteric arteries (6) and applied this technique to rats with spontaneous hypertension (7). The plasma membrane-enriched fraction of mesenteric arteries from hypertensive rats displayed some enhanced enzymatic activities similar to those found in the plasma membrane fraction of aorta from hypertensive rats, but the ATP-dependent Ca^{2+} accumulation by the plasma membrane fraction of mesenteric artery was increased (7) as opposed to the decreased ATP-dependent Ca^{2+} accumulation by aortic plasma membrane (5). More recently, we have found that the mesenteries which were included with the arterial smooth muscle as well as the adhering fat cells introduced a small but serious contamination in our previous membrane preparations (8, 9).

In the present communication, we report reevaluation of some properties of the plasma membrane isolated from mesenteric arteries of hypertensive rats using an improved membrane isolation procedure (9). We propose to determine: (a) Whether the previously observed enhancement of ATP-dependent Ca^{2+} accumulation by plasma membrane vesicles of mesenteric arteries in the hypertensive state truly reflects a difference in the mechanisms controlling contraction and relaxation of small and large arteries in hypertensive animals as suggested previously (7) or if it is a consequence of inclusion of plasma membrane from other cells. (b) Whether the alterations of biochemical parameters observed in arteries of spontaneously hypertensive animals can be accounted for by use of inappropriate control animals. We further extend this study to investigate whether changes of these biochemical properties of arterial plasma membrane also occur in rats with experimental hypertension induced by deoxycorticosterone-salt treatment as in these with genetic hypertension.

Animals. Three- to four-month-old Kyoto-Wistar strain of male spontaneous hypertensive rats (SHR)[1]

[1] The abbreviations used are: SHR, spontaneous hypertensive rats; KWR, Kyoto-Wistar strain; NWR, regular Wistar strain; DOC, deoxycorticosterone; DHR, DOC-induced hypertensive rats; DNR, DOC-treated normotensive rats.

This work was supported by the Canadian Heart Foundation.

0026-895X/80/010137-04$02.00/0

were ootained from Ayerst Laboratories (Montreal, Canada) and were used as breeding stock for brother-sister mating in our laboratory. Age-matched normotensive rats of Kyoto-Wistar strain (KWR) and regular Wistar strain (NWR) were purchased from Canadian Breeding Farm (Montreal, Canada) and Woodlyn Farm (Ontario, Canada), respectively. Ten-week-old male Woodlyn Wistar rats were also used for the studies of arterial plasma membrane after deoxycorticosterone (DOC) treatment. In one set of experiments, the NWR rats received unilateral nephrectomy, 1% sodium chloride in their drinking water, and subcutaneous injection of 0.5 ml of 10 mg/ml DOC dissolved in sesame oil, twice a week for 4–6 weeks to induce hypertension and were designated DOC-induced hypertensive rats (DHR). Corresponding control rats (DHR_c) for this set of experiments were similarly treated except that DOC injection was replaced by injection of equal volume of sesame oil. In another set of experiments, the rats received only DOC without unilateral nephrectomy or saline water, designated as DOC-treated normotensive rats (DNR) and the corresponding control rats (DNR_c) received only sesame oil by subcutaneous injection.

Blood pressure measurement. Systolic blood pressures of most of the rats used in this work were measured regularly and on the day before killing using the tail-cuff compression method. Averaged value of four or five readings reproducible within 10 mm Hg was taken as the systolic blood pressure of the rat.

Membrane preparations and analytical methods. Preparation of subcellular membrane fractions from mesenteric arteries of 10–12 normotensive and hypertensive rats was as previously described (9). The protein content of membrane fractions was estimated by the method of Lowry (10). Alkaline phosphatase activity was determined by the release of *p*-nitrophenol from *p*-nitrophenyl phosphate (11).

^{45}Ca accumulation studies were carried out by Millipore filtration technique as described previously (7). The incubation mixture contains 100 mM KCl, 5 mM ATP, 100 μM $CaCl_2$ labeled with 0.2–0.4 μCi/ml $^{45}CaCl_2$ and buffered with 50 mM imidazole to pH 7.0 at 37°. The concentration of free calcium in this incubation medium was calculated to be 17 μM (6). For ATP-independent Ca^{2+} accumulation, ATP was omitted and $CaCl_2$ concentration was 20 μM. The reaction time was 10 min unless otherwise indicated. Calcium accumulation at this time was at a plateau level. All filters (25-mm diameter, 0.45-μm pores, Matheson-Higgins Co.) were dissolved in 10 ml of Bray's solution (12) and counted to at least 2% accuracy.

Statistical analysis of the data which are expressed as mean ± standard deviation were done by Student's *t* test at a significance level of 0.05.

Body weight, blood pressure, and yield of mesenteric arteries. Table 1 shows that all the SHR used have sustained elevated blood pressure in the range of 160–180 mm Hg. Although a small portion of KWR had blood pressure near 140 mm Hg, only those with blood pressure less than 120 mm Hg were used for controls. The body weight of SHR was significantly less than that of either strain of normotensive control, whereas the weight of

TABLE 1

Body weight, blood pressure, tissue weight, and plasma membrane protein content of hypertensive rats and corresponding control rats

Rat used	Body weight	Blood pressure	Wet tissue weight[a]	Plasma membrane protein[b]
	(g)	*(mm Hg)*	*(g/kg)*	*(mg/g)*
NWR (4)[c]	398.8 ± 34.3	120	0.40 ± 0.02	0.39 ± 0.07
KWR (3)	400.3 ± 20.5	120	0.38 ± 0.01	0.30 ± 0.04
SHR (6)	282.5 ± 28.9[d]	160–180	0.46 ± 0.03[d]	0.46 ± 0.16
DHR_c (4)	407.5 ± 25.3	120	0.34 ± 0.05	0.49 ± 0.12
DHR (4)	417.5 ± 11.9	160–180	0.73 ± 0.12[d]	0.47 ± 0.11
DNR_c (4)	402.1 ± 35.5	120	0.37 ± 0.02	0.31 ± 0.06
DNR (4)	401.3 ± 25.6	120	0.36 ± 0.04	0.29 ± 0.06

[a] Wet tissue weight is expressed as grams of all arterial muscles per kilogram of total body weight of rats used.

[b] Plasma membrane protein is expressed as milligrams of total plasma membrane protein per gram of total arterial muscles used.

[c] Numbers of parenthesis indicate number of separate experiments performed using 10–12 rats each.

[d] Significantly different from control values ($p < 0.05$).

mesenteric arteries of the SHR after removing the veins, mesenteries, and fat tissues was, on the other hand, slightly and significantly more than that of the controls. However, the protein content of plasma membrane fractions normalized to the same tissue wet weight was not significantly different in SHR and control arteries.

The body weight of DHR and the corresponding control rats was not significantly different but the total wet weight of isolated arteries of DHR was much higher than that of the control rats. The DHR rats started developing hypertension at the end of the second week of DOC treatment and reached established hypertension at the end of fourth week with blood pressure ranging between 160 and 180 mm Hg. Prolonged treatment with DOC did not further elevate the blood pressure but caused increasing mortality due to complications of the regimen. A small number of rats (approximately 10%) which never developed blood pressure higher than 140 mm Hg during the entire period of DOC treatment were not used.

The rats (DNR) treated with deoxycorticosterone alone but neither uninephrectomized nor given saline for drinking water did not develop hypertension. Similar to DHR, the body weight of DNR was not significantly altered by the deoxycorticosterone treatment. But unlike DHR, the wet tissue weight of arteries of DNR was not significantly different from that of the controls.

Enzymatic activities and calcium accumulation by plasma membrane fractions. The alkaline phosphosphatase activity and Ca^{2+} accumulation by plasma membrane fractions isolated from carefully trimmed mesenteric arteries of hypertensive rats and corresponding controls are shown in Fig. 1. Both SHR and DHR plasma membrane fractions possess common features, i.e., increased alkaline phosphatase activity and decreased Ca^{2+} accumulation in the presence of ATP. Ca^{2+} accumulation in the absence of ATP on the other hand remained unaltered in all of these groups. This suggests that only the energy-dependent Ca^{2+} accumulation by the plasma membrane fraction is altered in hypertension. The reciprocal changes of alkaline phosphatase activity and ATP-dependent Ca^{2+} accumulation in these two types of hy-

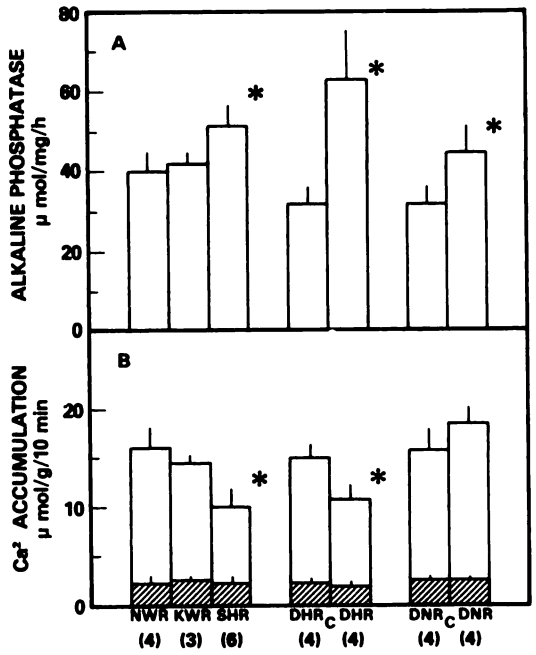

FIG. 1. *Alkaline phosphatase activity (A) and calcium accumulation (B) by plasma membrane fraction isolated from hypertensive rats*

Experimental conditions were as described in the text. The shaded columns represent the values of Ca^{2+} accumulation in the presence of 20 μM free calcium and in the absence of 5 mM ATP. Values in parentheses indicate the number of experiments. *, Significantly different from control values ($p < 0.05$).

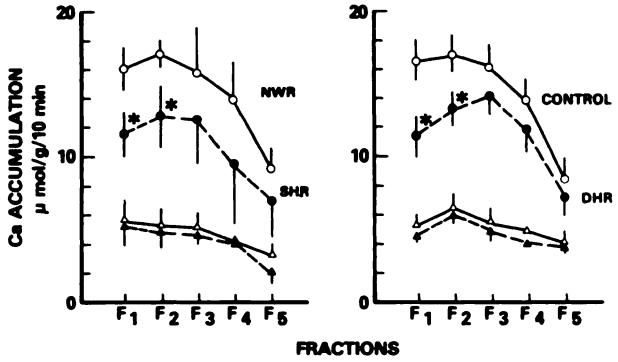

FIG. 2. *Calcium accumulation by membrane fractions recovered from discontinuous sucrose density gradient*

The membrane composition of these fractions (lightest fraction F_1 to heaviest fraction F_5) has previously been described in detail (8, 9). X 537A was dissolved first in 95% ethanol and then added as aliquots to the reaction medium to a final concentration of 10 μM. Inclusion of equal volume of ethanol in the medium did not significantly alter the calcium accumulation. Each data point represents the mean of values from three to five separate experiments with standard deviation as vertical bars. Data points without vertical bars represent the average of duplicate values in a single experiment. Closed symbols are used for hypertensive groups and open symbols are used for control groups. Triangular symbols are data obtained in the presence of X 537A. *, Significantly different from control values ($p < 0.05$).

pertension is not observed in DNR, whose blood pressure is not affected by DOC treatment alone, although such treatment of DOC alone caused some increase of alkaline phosphatase activity as well as slight but not significant increase of ATP-dependent Ca^{2+} accumulation. This small increase of alkaline phosphatase activity, however, cannot fully account for the increase of alkaline phosphatase activity in DHR.

We have also examined 5′-nucleotidase activity, another marker for plasma membrane, as well as cytochrome *c* oxidase and NADPH–cytochrome *c* reductase activities, markers for mitochondria and endoplasmic reticulum (9), respectively, in various fractions of hypertensive and control arteries, but no significant difference was observed (data not shown) indicating that there is no relative alteration of distribution of membrane fractions or differential contamination by other membrane fragments in arterial plasma membranes isolated from hypertensive and normotensive groups.

Ca^{2+} accumulation by other fractions from sucrose density gradient. The ATP-dependent Ca^{2+} accumulation by fractions obtained from the subfractionation of microsomal membranes on a sucrose density gradient (see Ref. (9)) and the effect of a calcium ionophore, X 573A are shown in Fig. 2. The difference in Ca^{2+} accumulation between fractions of hypertensive and normotensive control groups was more prominent in lighter fractions and became small and less significant toward heavier fractions, indicating that the defective Ca^{2+} handling lies in the plasma membrane fraction. X 573A reduced the ATP-dependent Ca^{2+} accumulation to the same level in both groups of either type of hypertension. However, the Ca^{2+} accumulation by these fractions in the absence of ATP was not significantly different from the corresponding control values in either hypertensive group and X 537A did not alter the Ca^{2+} accumulation in the absence of ATP. This seems to suggest that the ATP-dependent Ca^{2+} transport across the vascular plasma membranes rather than the Ca^{2+} binding was altered in hypertension.

The results reported in this communication indicate that some biochemical properties of the plasma membranes isolated from small arteries of SHR and DHR were similarly altered. The alterations include (a) increased total protein content which is proportional to the increased arterial wet weight; (b) enhanced alkaline phosphatase activities; and (c) reduced Ca^{2+} accumulation in the presence of ATP. These findings clarified some of the problems associated with our previous studies concerning specifically the enhanced ATP-dependent Ca^{2+} accumulation by the plasma membrane fraction isolated from the mesenteric arteries of SHR, and the strains of normotensive controls rats that were different from that of SHR.

The discrepancy between our present finding of a reduced ATP-dependent Ca^{2+} accumulation and the previous finding of an increased ATP-dependent Ca^{2+} accumulation (7) by plasma membrane fractions isolated from mesenteric arteries of SHR is most likely due to membrane contamination derived from the enormous amount of adhering mesenteric fat tissue, especially in view of our preliminary finding that the plasma membrane fraction isolated from such mesenteric fat tissues of SHR showed marked increase of ATP-dependent accumulation.[2] This is further justified by our similar find-

[2] Kwan, C.-Y., L. Belbeck and E. E. Daniel, unpublished observations.

ing of a reduced ATP-dependent Ca^{2+} accumulation by plasma membrane enriched fraction isolated from aorta of SHR (5), which contains little or practically no adhering fat tissues. Besides, the plasma membranes of mesenteric arteries of DHR show reduced ATP-dependent Ca^{2+} accumulation as well.

The vascular plasma membrane abnormalities of SHR cannot be attributed to the use of different genetic strains of normotensive control rats as these biochemical parameters studied are very similar between NWR and KWR. This is also supported by the very similar findings observed in DHR since the same strain of rats was employed for the controls.

Alteration of vascular wall has received considerable attention as one of the various factors contributing to the increase of total peripheral resistance of blood vessels in hypertension (13). The vascular changes have been attributed to the structural changes (increased wall thickness to lumen diameter ratio) as proposed by Folkow (14, 15) or to the functional change of ion transport across vascular cell membranes (5, 16, 17). In genetic as well as experimental hypertension, the increase of arterial wet weight paralleled by the increase of total arterial plasma membrane protein content, indicating vascular hypertrophy, may result from the structural changes of the arterial wall, while the reduced Ca^{2+} transport across the vascular plasma membrane, indicating functional abnormality, may be associated with a defect in relaxation of vascular smooth muscle in hypertension (4, 17). The link between enhanced alkaline phosphatase activity and hypertension is not clear because the physiological function of this enzyme, whether soluble or membrane-bound, is not understood in blood vessels (18). However, it has been speculated that alkaline phosphatase may be involved in the control of Ca^{2+} binding or transport via dephosphorylation of membrane proteins (7, 19). More effort is needed to characterize and define the role of such alkaline phosphatase in vascular smooth muscle function.

The present finding of similar alterations of vascular smooth muscle plasma membrane in both genetic hypertension and DOC/salt-induced experimental hypertension lead us to further consider the following questions: (a) Are these plasma membrane abnormalities common to most forms of hypertension? (b) What is the cause–effect relationship between hypertension and these membrane abnormalities? We are currently investigating these problems.

ACKNOWLEDGMENT

We thank Ms. K. Wong for her excellent technical assistance.

REFERENCES

1. Cohen, M. L. and B. A. Berkowitz. Decreased vascular relaxation in hypertension. *J. Pharmacol. Exp. Ther.* **196:** 396–406 (1976).
2. Aoki, K., K. Yamashita and K. Hotta. Calcium uptake by subcellular membranes from vascular smooth muscle of spontaneously hypertensive rats. *Jap. J. Pharmacol.* **26:** 624–627 (1976).
3. Furuta, Y. Studies on sodium and calcium contents of cardiovascular tissue in experimental hypertension. *Jap. Circ. J.* **41:** 19–28 (1977).
4. Field, F. P., R. A. Janis and D. J. Triggle. Aortic reactivity of rats with genetic and experimental renal hypertension. *Can. J. Physiol. Pharmacol.* **50:** 1072–1079 (1972).
5. Wei, J. W., R. A. Janis and E. E. Daniel. Calcium accumulation and enzymatic activities of subcellular fractions from aortas and ventricles of genetically hypertensive rats. *Circ. Res.* **39:** 133–140 (1976).
6. Wei, J. W., R. A. Janis and E. E. Daniel. Isolation and characterization of plasma membrane from rat mesenteric arteries. *Blood Vessels* **13:** 279–292 (1976).
7. Wei, J. W., R. A. Janis and E. E. Daniel. Studies on subcellular fractions from mesenteric arteries of spontaneously hypertensive rats. Alterations in both calcium uptake and enzyme activities. *Blood Vessels* **13:** 293–308 (1976).
8. Daniel, E. E., C.-Y. Kwan, M. A. Matlib, D. Crankshaw and A. Kidwai. Characterization and Ca^{2+} accumulation by membranes fractions from myometrium and artery, in *Excitation–Contraction Coupling in Smooth Muscle* (R. Casteels *et al.*, eds.). Elsevier, Amsterdam, 181–188 (1977).
9. Kwan, C.-Y., R. Garfield and E. E. Daniel. An improved procedure for the isolation of plasma membranes from rat mesenteric arteries. *J. Mol. Cell. Cardiol.* **11:** 639–659 (1979).
10. Lowry, O. H., N. J. Rosebrough, A. L. Farr and R. J. Randall. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275 (1951).
11. Bessey, O. A., O. H. Lowry and M. J. Brock. A method for the rapid determination of alkaline phosphatase with five cubic millimeter of serum. *J. Biol. Chem.* **164:** 321–329 (1946).
12. Bray, G. A. A simple efficient liquid scintilation for counting aqueous solutions in a liquid scintillation counter. *Anal. Biochem.* **1:** 279–285 (1960).
13. Davis, J. O. The pathogenesis of chronic renovascular hypertension. *Circ. Res.* **40:** 439–444 (1977).
14. Folkow, B. The homodynamic consequences of adaptive structural changes of the resistance vessels in hypertension. *Clin. Sci.* **41:** 1–2 (1971).
15. Folkow, B., M. Hallback, J. V. Jones and M. Sutter. Effects of noradrenaline on consecutive vascular segments at low or normal calcium concentrations in control and spontaneously hypertensive rats. *Clin. Sci. Mol. Med.* **51:** 53–55 (1976).
16. Jones, A. W. and R. G. Hart. Altered ion transport in aortic smooth muscle during deoxycorticosterone acetate hypertension in the rat. *Circ. Res.* **37:** 333–341 (1974).
17. Levy, J. V. Differences in papaverine inhibition of prostaglandin-induced contraction of aortic strips from normal and spontaneously hypertensive rats. *Eur. J. Pharmacol.* **25:** 117–120 (1974).
18. Fernley, H. N. Mammalian alkaline phosphatase, in *The Enzymes*, Vol. 4 (P. D. Boyer, ed.). Academic Press, New York, 417–447 (1971).
19. Twietmeyer, T. A., R. C. Bhalla and J. A. Maynard. Acid and alkaline phosphatase activities and calcium transport in aortic smooth muscle from DOCA hypertensive rats. *J. Mol. Cell. Cardiol.* **10:** 1131–1140 (1978).

Send reprint requests to: Dr. Chiu-Yin Kwan, Department of Neurosciences, McMaster University, 1200 Main St., West, Hamilton, Ontario L8S4J9, Canada.