Differential Response of Hamster Cheek Pouch Microvessels to Vasoactive Stimuli during the Early Development of Hypertension

ROGER L. CLICK, JOSEPH P. GILMORE, AND WILLIAM L. JOYNER

SUMMARY Arteriolar responses in the hamster cheek pouch to direct microapplications of norepinephrine (NE), angiotensin II (A II), and potassium chloride (KCI) were investigated during the early developmental phase of hypertension. After determination of arterial pressure in 12 hamsters, control luminal and wall diameters and the response of arterioles (second order branching, 28-60 μm diameters) to microapplications of NE (0.5, 1.0, 5.0 ng), A II (0.5 ng), KCI (22.2 ms) and the vehicle (Tris-buffered Ringer's solution) were determined. Then using a figure-eight ligature around each kidney hypertension was induced in nine animals, and three were sham-operated. Measurements were repeated on the same hamsters 4, 8, and 13 days later. Four of the nine animals developed a sustained hypertension (HT-S) and the remaining five developed a transient hypertension (HT-T), only on day 4. The sham-operated hamsters remained normotensive. The arteriolar response to KCI was increased significantly in the HT-S group on days 4 and 8 whereas the arteriolar response to NE and A II was increased significantly on days 8 and 13. There were no significant differences in the arteriolar responses of either the HT-T or normotensive group to any agent at any time. Furthermore, there were no significant changes in arteriolar wall/lumen ratios for any group at any time. Thus, the transient nature of the arteriolar response to potassium combined with the delayed increase in the arteriolar response to NE and A II implies that there are two vascular phases associated with the early development of hypertension in this model. The first phase may be an ionic alteration which in turn may initiate the second or humoral phase.

ALTERATIONS that can result in an increased peripheral resistance in hypertension may involve various components: (1) neurogenic, an altered autonomic output to the peripheral circulation, (2) hormonal, increased levels of vasoconstrictor or decreased levels of vasodilator substances, (3) local, changes in the vascular smooth muscle-receptor complex which lead to increased sensitivity of the blood vessels, and (4) structural, hypertrophy of the vascular smooth muscle concomitant with a decrease in luminal diameter of the arterioles. All of these factors may contribute to an increase in peripheral resistance, but the local and structural components have gained the strongest emphasis (Brody and Zimmerman, 1976).

Until recently, most conclusions about the microcirculation during hypertension have been limited to the use of whole organ (Collis and Alps, 1975; Finch and Haeusler, 1974; Lais et al., 1974), isolated vascular segment (Folkow et al., 1970; McQueen, 1956; Redleaf and Tobian, 1958), and whole animal (Finch, 1971; Okamoto et al., 1966) preparations. Thus, changes which may have taken place at the microcirculatory level to alter peripheral vascular resistance were determined indirectly. More recently, various laboratories using spontaneously hypertensive rats (SHR) (Bohlen et al., 1977; Hutchins and Darnell, 1974) or renovascular models of hypertension (Click et al., 1977; Harris et al., 1975) have demonstrated that the structural characteristics (wall/lumen ratios) of the microvessels were not altered in the early stages of hypertension. However, arterioles (40-60 μm) of the hamster cheek pouch were shown to be more sensitive to norepinephrine in a renovascular model (Click et
al., 1977), whereas the number of patent arterioles in the cremaster muscle of the SHR has been reported to be decreased (Hutchins and Darnell, 1974).

Studies from this laboratory investigating microvascular alterations in the hamster cheek pouch preparation have described two stages of hypertension (Click et al., 1977). The first is an early phase (15 days) which is characterized by an increased arteriolar sensitivity to norepinephrine. The second is a later phase (70 days) which also is characterized by an increased arteriolar sensitivity to norepinephrine as well as increases in both venular sensitivity and arteriolar wall/lumen ratio.

The present study was designed to delineate the alterations in vascular sensitivity during the early phase of hypertension. Arteriolar responses to receptor-specific agents, norepinephrine and angiotensin II, and a nonspecific agent, potassium chloride, were followed in chronic preparations of the hamster cheek pouch at different times during the early development of hypertension.

Methods

Systolic arterial blood pressure (mean of three independent measurements) was determined indirectly using an inflation cuff and pulse transducer (Click et al., 1977) in 12 hamsters anesthetized with pentobarbital sodium (6 mg/100 g, ip). After blood pressure had been measured, a plastic chamber was inserted into the cheek pouch and the cheek pouch membrane exposed as previously described (Click et al., 1977). The hamster was placed on a heated stage under a Zeiss microscope (Collins Microscope Co.) and the cheek pouch membrane was suffused constantly with Tris-buffered Ringer’s solution maintained at 37°C. Following stabilization of the membrane (15 minutes), arterioles (38–60 μm internal diameter) that were in the same branching order of the microcirculation for each hamster were chosen for study. Control arteriolar wall and luminal diameters were measured with a Vicker’s image shearer. Thereafter, the arteriolar response to the local microapplication (3.35-μl volumes) of norepinephrine (0.5, 1.0, and 5.0 ng, Levophed-Winthrop), potassium chloride (22.2 mM), angiotensin II (0.5 ng Hypertensin-Ciba) and the vehicle (Tris-buffered Ringer’s) was determined by repeated measurements of the luminal diameter for 5 minutes after the application of each vasoactive agent. Thus, due to time limitations and refractoriness, only one dose of either potassium chloride or angiotensin II was used in these experiments. The order of application (as described above) and time course were maintained for all animals. These measurements were control (day 0) values.

After this procedure the chamber was removed and the outer skin sutured. In 17 hamsters a Grollman (Grollman, 1944) procedure, which is a figure-of-eight ligature tied around each kidney, was performed to induce hypertension (hypertensive group), and three animals were sham-operated (normotensive group). The hamsters were allowed to recover and the cheek pouch microcirculation again evaluated 4, 8, and 13 days later.

Statistical analysis of the data was completed using the Student’s paired t-test. Regression and F ratio analysis were used to calculate and compare slopes of the dose-response curves. P < 0.05 was considered statistically significant.

Results

Of the 17 Grollman hamsters, seven died prior to day 4 and one died prior to day 13. All sham-operated animals survived through day 13, and there was no significant change in arterial pressure (normotensive). In the Grollman group, four animals developed a sustained hypertension (hypertensive-S) through day 13 and five developed a transient hypertension (hypertensive-T) which lasted for 4–8 days.

Therefore, the data were analyzed for each day as three separate groups: hypertensive-S (n = 4), hypertensive-T (n = 5), and normotensive (n = 3). Figure 1 shows the blood pressures for each hamster in the three designated groups. The blood pressure for the hypertensive-S group on day 0 (first drug test) was 95 ± 6 mm Hg, for the hypertensive-T group 102 ± 2 mm Hg, and for the normotensive group 100 ± 5 mm Hg. By day 4 (second drug test),
The response of one hamster from the hypertensive-S group to norepinephrine, KCl, and angiotensin II applied to an arteriole on days 0, 4, 8, and 13 is shown in Figure 2. When blood pressure had increased from 99 mm Hg on day 0 to 136 mm Hg on day 4, the vasoconstrictor response to each of the vasoactive agents had increased. The percent increase from day 0 was greatest for KCl (54%), whereas the percent increases for norepinephrine and angiotensin II were 31% and 12%, respectively. On day 8, when blood pressure had increased to 134 mm Hg, the arteriolar response to each of the agents was similar to the response observed on day 4. By day 13, blood pressure had increased to 160 mm Hg and the response to angiotensin II was increased, compared to that on days 4 and 8. Also, there was a further increase in the arteriolar response to norepinephrine, whereas the response to KCl had returned to control values.

The response of the arterioles to norepinephrine, KCl, angiotensin II, and the vehicle for all groups at each time period is shown in Figure 3. On day 0, there was no significant difference in the response to any agent tested for any group. However, on day 4, the arteriolar response of the hypertensive-S group was increased significantly for KCl, compared to either the normotensive controls or the hypertensive-T group. Also, one response to norepinephrine (5.0 ng) in the hypertensive-S group was elevated. There was no significant difference between the responses of the arterioles to any agent for the hypertensive-T as compared to the normotensive group on day 4. On day 8, the arteriolar response of the hypertensive-S group to KCl, angiotensin II, and all doses of norepinephrine was increased significantly, compared to either the normotensive or hypertensive-T group. Also, the response to KCl was significantly less in the hypertensive-T group as compared to the normotensive group. On day 13, the response of the hypertensive-S group remained increased significantly compared to either normotensive or hypertensive-T groups for norepinephrine and angiotensin II. However, the response to KCl was not significantly different from the normotensive hamsters but was increased significantly when compared to the hypertensive-T group. Comparison of the values for the normotensive and hypertensive-T groups on day 13 showed no significant difference for any agent tested. When intra-
group comparisons were analyzed for the hypertensive-S animals on day 4 as compared to day 0, only the KCl response was increased significantly. However, when the values on day 8 were compared to those on day 0, there was a significant increase in the response of the arterioles to all agents. When the values on day 13 were compared to those on day 0, there was a significant increase in the response of the arterioles to both norepinephrine and angiotensin II, but there was no significant difference in the response to KCl. Similar comparisons for either the hypertensive-T or normotensive hamsters revealed that there were no significant differences in the arteriolar response to any agents. Finally, for all groups, the vehicle produced no significant changes (2-8% of the control diameter) in the arteriolar diameters.

Further analysis of the data was obtained by calculating the slope of the norepinephrine dose-response curves and statistically comparing these slopes for each group at days 0, 4, 8, and 13. There was no significant difference between the slopes of the norepinephrine dose-response curves for any group at any time period. Using the linear regression analysis, ED50's were calculated for each group at each time period as determined by the dose of norepinephrine required to produce a 50% change in luminal diameter. For the normotensive group at all periods, the ED50's ranged from 4.6 to 5.8 ng of norepinephrine and the ED50's for the hypertensive-T group ranged from 5.3 to 6.9 ng of norepinephrine on days 0, 8, and 13, while the ED50 decreased, but not significantly, to 2.7 on day 4. For the hypertensive-S group the ED50 steadily fell from day 0 to day 13 (4.3, 2.5, 1.7, and 0.5 ng of norepinephrine, respectively) but was not significantly decreased until day 8. Thus, this altered ED50, along with the parallelism of the norepinephrine dose-response curves, indicated an increased arteriolar sensitivity in the hamsters that developed a sustained hypertension (hypertensive-S group).

There was no significant difference in arteriolar wall/lumen ratios (0.182-0.198) for any group at any time period. Also, there was no significant difference in the luminal diameter (um ± se) of the arterioles at day 0 for the hypertensive-S (44 ± 2), hypertensive-T (47 ± 4), or the sham (47 ± 3) groups. These values were not altered significantly on days 4, 8, or 13 in any group.

Discussion

Using the Grollman procedure to produce the hypertensive state in hamsters, we found two distinct effects on arterial blood pressure during the early developmental period (1-2 weeks). In four of nine hamsters (44%), arterial blood pressure was elevated on day 4 and remained elevated throughout the experimental period, whereas in five of nine animals (56%) arterial blood pressure was elevated early on day 4 but returned to normal levels by day 8. Using the same technique in hamsters, Ströia et al. (1954) obtained similar results, but the proportion of hamsters that were transiently hypertensive (19%) was lower. Also, the time course for the development of hypertension in their animals was prolonged (10-20 days). Click et al. (1977) have shown that if blood pressure was not elevated by day 13, the animals would not become hypertensive.

The increased response to potassium, norepinephrine, and angiotensin II occurred only in those animals that developed a sustained hypertension. Also, at no time was there any change in arteriolar wall/lumen ratios. In these hamsters, the arteriolar response to potassium was increased (122%) on day 4 and remained elevated through day 8. Thereafter, the arteriolar response to potassium returned to control levels, whereas the increased response to norepinephrine and angiotensin II which was manifested on day 8 did not wane. An increased vascular response to potassium has been observed in other hypertensive models (Bohr and Sitrin, 1970; Collis and Alps, 1975; Field et al., 1972; Haeusler and Haefely, 1970). Haeusler and Haefely (1970) observed an increased response to potassium and norepinephrine in the perfused mesentery of rats 6-8 weeks after a Grollman procedure. However, Collis and Alps (1975) reported a delayed increase in the response to potassium which occurred 4-6 weeks after the Grollman procedure, whereas no change was observed at 2 weeks. Although the present study confirms the observation of the association of an altered potassium response with the development of hypertension, the differences in the time course of the response reported here and elsewhere may be due to several factors including: (1) the model, (2) the means of administering the potassium, or (3) the mode of assessing the response. Thus, the transient nature of the potassium response and the subsequent increased response to norepinephrine and angiotensin II implies that there are two vascular phases associated with the early development of hypertension. The first phase, as expressed by an increased response to potassium, may be due to an ionic and/or electrogenic alteration which changes the membrane potential in vascular smooth muscle. These events may initiate the second or humoral phase. The apparent disappearance of the ionic and/or electrogenic component while the increased response to other agents continues would lead one to speculate that some other mechanisms might be potentiating the vascular response during the second or humoral phase. In any event, the difference in the potassium response for these two groups (hypertensive-sustained vs. hypertensive-transient) strengthens the argument that potassium may play a dominant role during the early development of hypertension and indicates that full expression of an ionic alteration may be necessary prior to the initiation of the humoral phase.

The initial phase as expressed by an increased vascular response to potassium may be due to al-
terations in the electrophysiological properties of vascular smooth muscle (Carrier and Shibata, 1977). Jones (1974) noted a decreased ability of blood vessels from SHR to accumulate potassium and extrude sodium; thus these alterations may be reflected in a partial depolarization of the membrane and a decreased membrane potential. If the vascular smooth muscle is stimulated by vasoactive substances, the results would be an increased response. Hermansmeyer (1976) found that when electrogenic ion transport was suppressed by low temperature, the SHR smooth muscle cells have a less negative membrane potential than normal. He concluded that the altered electrogenic ion transport of vascular smooth muscle in the SHR is implicated in the increased response to vasoactive substances. Thus, the altered potassium response that we observed may reflect an early alteration in membrane potential which, after a period of time, manifests itself as an altered response to all vasoactive substances.

In many forms of hypertension the renin-angiotensin system is altered (Page and McCubbin, 1968). The present results indicate an altered response of the vasculature to angiotensin II during the development of hypertension in this model. In addition to its direct action on the vasculature, angiotensin II may enhance the vasoconstrictor effect of norepinephrine at synaptic junctions by: (1) inhibiting neuronal uptake of released norepinephrine, (2) augmenting the release of norepinephrine per impulse, (3) releasing norepinephrine directly, or (4) enhancing the biosynthesis of norepinephrine (Starke, 1977). These actions may be mediated by alterations in intracellular calcium (Collis and Alps, 1974). Angiotensin II has been shown also to enhance the sympathetic vasoconstrictor response at the peripheral (McCubbin and Page, 1963) or central level (Somlyo and Somlyo, 1970). The altered response to local application of angiotensin II even though angiotensin II levels may be elevated seems contradictory in light of the tachyphylaxis observed in this vascular bed. However, an increased response to angiotensin II has been observed in other hypertensive models in which the activity of the renin-angiotensin system is increased (Collis and Alps, 1975). Furthermore, Baum and Shrophshire (1967) observed an increased response to exogenous angiotensin II in the perfused hindquarter preparation of rats in which hypertension was induced by the Grollman procedure. Thus, this information, along with data showing that an angiotensin antagonist (saralasin) lowered arterial blood pressure at 4, 13, and 60 days after the induction of hypertension induced by the Grollman technique to a greater extent than in a comparable sham group, indicates that the Grollman model for hypertension may be a form of high renin hypertension (Click, 1977).

This study confirms our previous observations showing an increased vascular sensitivity paralleling changes in blood pressure without an increase in vascular wall thickness. However, it is clear that there are early changes in vascular sensitivity in this form of hypertension and that these changes are associated with temporal and differential alterations in the response of microvessels to vasoactive stimuli.

Acknowledgments

Excellent technical assistance was provided by Nargis Wahab, and Dr. Kashinath D. Patil aided in the statistical analysis of the data.

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DIMINISHED OUABAIN RESPONSE IN AGED MYOCARDIUM/Gerstenblith et al.

Gary Gerstenblith, Harold A. Spurgeon, Jeffrey P. Froehlich, Myron L. Weisfeldt, and Edward G. Lakatta

SUMMARY We studied the effect of advanced age on the response to paired pacing and on the relationship between ouabain-induced inotropy and inhibition of \((\text{Na} + \text{K})\)-ATPase in hearts from young adult and senescent rats. In isometric trabeculae carneae, control values of developed tension \((\text{DT})\) and maximal rate of tension development \((\text{dT/dt})\) were not age-related. There was no age-related difference in the response to extrasystolic potentiation at prematurity intervals from 400 msec to the mechanical refractory period. The maximal response occurred at a prematurity interval of 200 msec and was above 200\% of control. The inotropic response to ouabain occurred over a concentration range from \(2 \times 10^{-6}\) to \(6 \times 10^{-5}\) M. DT and dT/dt in muscles from the young adult group exhibited a greater response than those from the senescent group; e.g., at \(6 \times 10^{-5}\) M both parameters were approximately four times greater in the former group. There was no age-dependent difference in ouabain-induced enzyme inhibition, which occurred over the same concentration range as did ouabain-induced alterations in mechanical function. These data indicate that paired pacing, but not ouabain, results in similar increases in inotropy and therefore similar increases in myoplasmic calcium concentration in young adult and aged rat myocardium. The age-dependent difference in ouabain responsiveness appears to be related to an age-associated alteration in a step other than enzyme inhibition linking ouabain binding to increased myoplasmic calcium levels.

Circ Res 44: 517-523, 1979

PREVIOUS WORK from this laboratory has demonstrated that cardiac muscle isolated from senescent as compared to that from adult rats exhibits a diminished inotropic response to catecholamines but a normal maximal inotropic response to an increased extracellular calcium concentration, \([\text{Ca}^{2+}]\). (Lakatta et al., 1975). This finding suggests that the ability of the myofibrils to generate maximal force is not affected by age, but that sarcolemmal or intracellular structures and events that increase calcium delivery to the myofibrils in response to catecholamines may be altered with age. Paired pacing and ouabain are other inotropic interventions whose mechanisms of action are reported to be mediated by increasing the calcium concentration available to bind with troponin. Although considerable information is available concerning the effect of developmental changes on the volume of distribution of ouabain, and on the inotropic, electrophysiological, and biochemical responses to cardiac glycosides, (Langer et al., 1975; Akera et al., 1972; Inturrisi and Papaconstantinou, 1974; Rosen et al., 1975; Kelliher and Roberts, 1976; Berman et al., 1977; Glantz et al., 1976; Scott et al., 1971), the effect of advanced age on these responses has not been described. Adult rat myocardium generally has been reported to be insensitive to ouabain (Langer et al., 1975; Scott et al., 1971; Repke, 1963; Detweiler, 1967). However, preliminary experiments in our laboratory as well as in others have shown that under appropriate conditions a mechanical response to ouabain may occur (Masuoka and Saunders, 1950; Gerstenblith et al., 1975).
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R L Click, J P Gilmore and W L Joyner

Circ Res. 1979;44:512-517
doi: 10.1161/01.RES.44.4.512

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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