

METHODS

SHR and WKY rats weighing 200-300 g were killed by a stroke on the head. The portal vein was quickly dissected out, and 5 mm strips of the vessel was mounted in a 40 ml bath. The isometric tension in the preparation was measured with model FT03 Grass transducer and the trace was recorded on a polygraph. In each experiment atleast one preparation of SHR portal vein was mounted with a control preparation of WKY.

Solutions : Normal Ringer's solution contained in mM: 140 NaCl, 6 KCl, 1.5 CaCl₂, 1.5 NaH₂PO₄, 1 MgCl₂ and 10 glucose. The pH of all solutions was kept at 7.4.

Concentrated salt solutions were prepared by total replacement of the NaCl in the normal Ringer's solution with the required salt keeping all other constituents constant. Hence the net concentration of the salt was 140 mM.

Drugs : NE (Arterenol bitartrate) and all other chemicals were obtained from Sigma.

Experimental protocol : The portal vein strips were immersed in 40 ml of normal Ringer's solution at 37°C under a starting tension of 1 g. The preparation was left to stabilize for one hour before any experimental solution was introduced. Wherever appropriate the vessel was activated with 1 µM NE or 26 mM K⁺ to produce base line tone. Certain measured volumes of the concentrated stock salt solutions were then added to the bath to achieve the required concentration of the salt in the bath without changing the osmolarity. The salt solution was left for 5 min and then washed with the control solution for atleast 5 min before another salt was applied.

Analysis of traces : Tone variations due to the salt are expressed as means ± standard error of the mean of their relative values compared to the presalt tone. The significance of tone deviations from unity (presalt tone) and of SHR as compared to WKY rats are evaluated by Student's unpaired t-test. P values <0.05 are considered significant. Recovery rate from acid load is expressed as follows (13).

The % recovery from ammonium constriction (acid load) is taken as

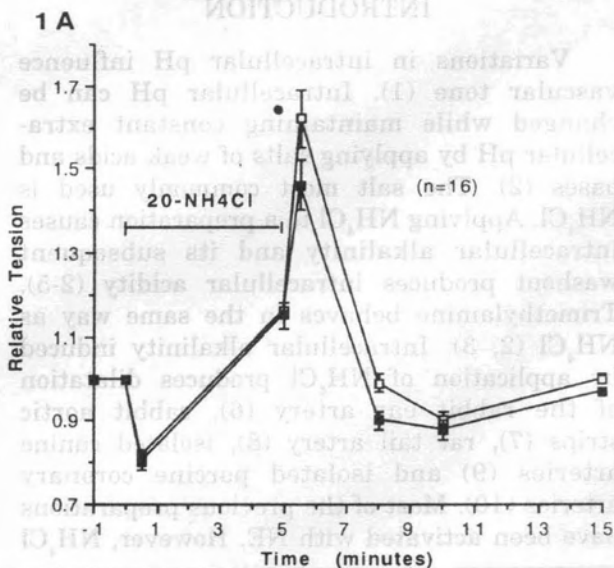
$$\frac{c-d}{c-1} \times 100$$

Where

- c = maximum tone reached by ammonium washout,
d = tone measured 4 min after 'c' and
1 = presalt tone

RESULTS

Application of 20 mM NH₄Cl dilated NE activated portal vein preparations of both SHR and WKY rats (Fig. 1A). Subsequent washout of the salt produced constriction in both preparations. The same qualitative results were obtained in nonactivated and 26 mM K⁺ activated and preparations (Fig. 1B, 1C). In all of these three traced the maximum dilatation produced by NH₄Cl was obtained in the first half a minute of application, while the maximum washout tension rise was attained within the first min. Recovery of tone towards prepulse level occurred after these maximum points of reduction and increase in tension. Trimethylamine produced the same qualitative results as NH₄Cl in both



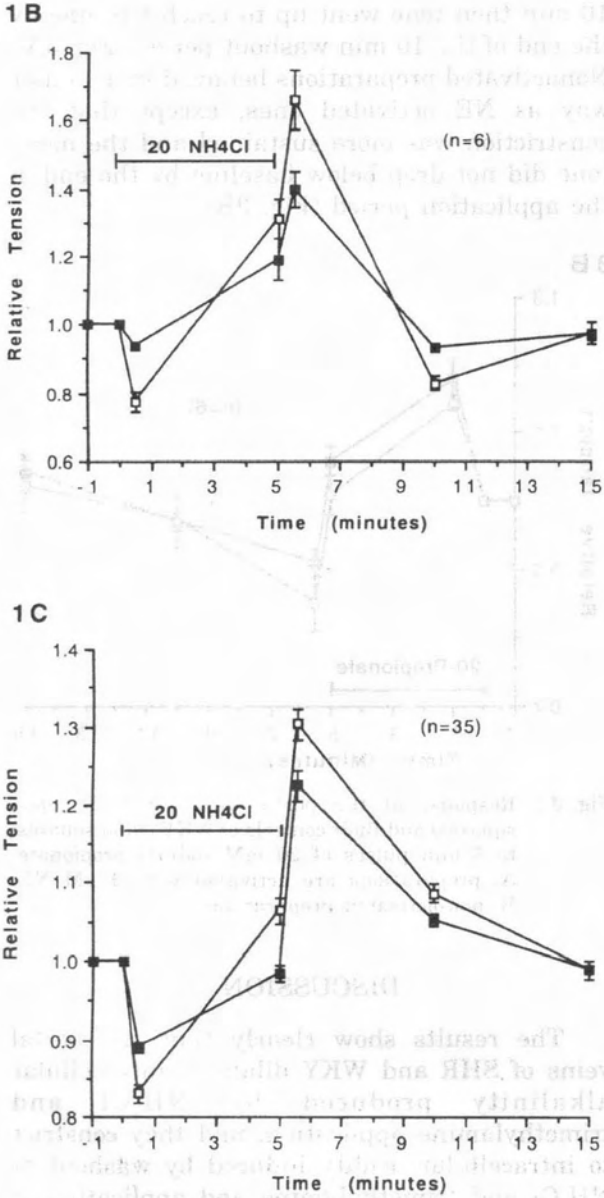


Fig. 1 : Response of the portal vein preparations of SHR (open squares) and their controls of WKY (solid squares) to 5 min pulses of 20 mM NH₄Cl. A: preparations are activated with 1 μM NE. B: non-activated preparations. C: preparations are activated with 26 mM K⁺.

preparations with and without NE activation (Fig. 2A and 2B). The rate of recovery from NH₄Cl and trimethylamine washout constriction was significantly slower in the SHR portal vein

preparations than in the WKY ones (Table I). This reduction was greater and more significant in the NE activated preparations.

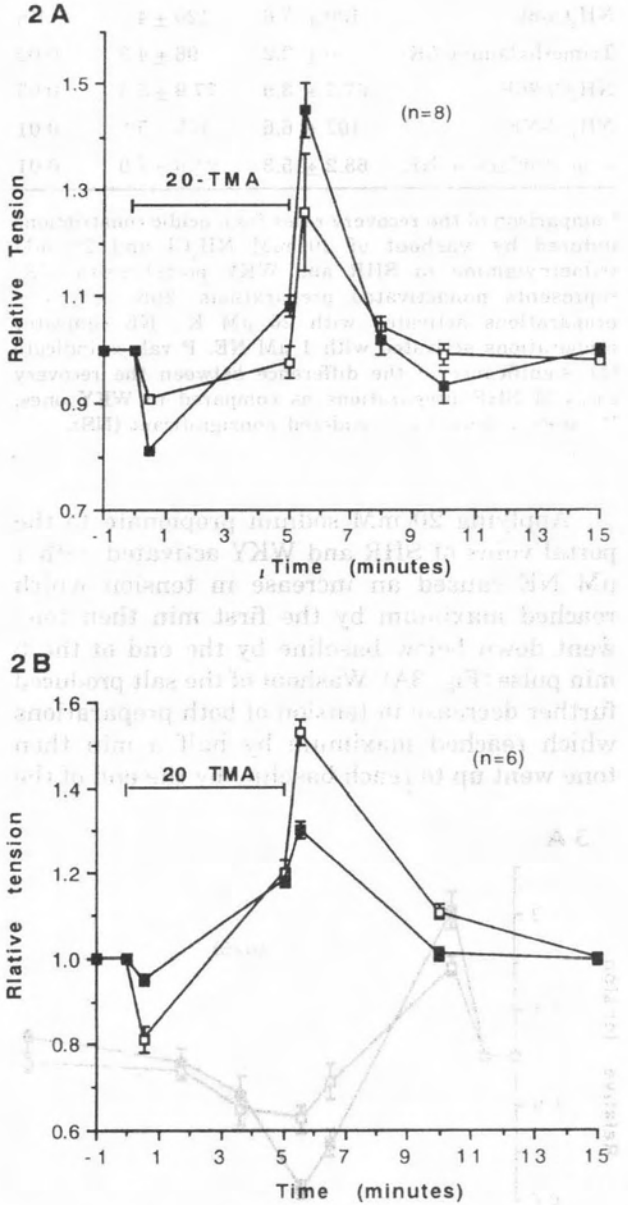


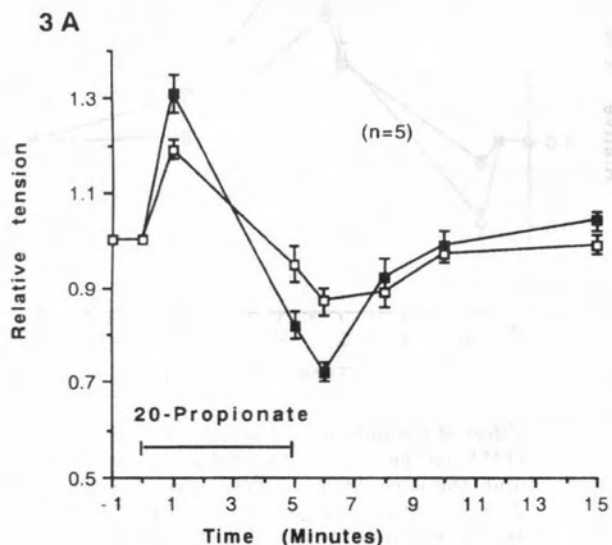
Fig. 2 : Effect of 5 min pulses of 20 mM trimethylamine (TMA) on the portal veins of SHR (open squares) and their controls of WKY (solid squares). A: preparations are activated with 1 μM NE. B: non-activated preparations.

TABLE 1 : Recovery rates from acidic loads of SHR and WKY portal veins.

Treatment	%Recovery from acidic load		
	SHR	WKY	P value
NH ₄ Cl-6k ⁺	130 ± 7.6	120 ± 4	NS
Trimethylamine-6K ⁺	80 ± 3.2	96 ± 4.9	0.02
NH ₄ Cl-26K ⁺	67.5 ± 3.9	77.9 ± 3.4	0.05
NH ₄ Cl-NE	102 ± 6.6	125 ± 5.6	0.01
Trimethylamine-NE	63.2 ± 15.3	91.8 ± 7.9	0.01

Comparison of the recovery rates from acidic constrictions induced by washout of 20 mM NH₄Cl and 20 mM trimethylamine in SHR and WKY portal veins. 6K⁺ represents nonactivated preparations. 26K⁺ indicates preparations activated with 26 μM K⁺. NE indicates preparations activated with 1 μM NE. P values indicate the significance of the difference between the recovery rates of SHR preparations as compared to WKY ones. P values > 0.05 are considered nonsignificant (NS).

Applying 20 mM sodium propionate to the portal veins of SHR and WKY activated with 1 μM NE caused an increase in tension which reached maximum by the first min then tone went down below baseline by the end of the 5 min pulse (Fig. 3A). Washout of the salt produced further decrease in tension of both preparations which reached maximum by half a min then tone went up to reach baseline by the end of the



10 min then tone went up to reach baseline by the end of the 10 min washout period (Fig. 3A). Nonactivated preparations behaved in a similar way as NE activated ones, except that the constriction was more sustained and the mean tone did not drop below baseline by the end of the application period (Fig. 3B).

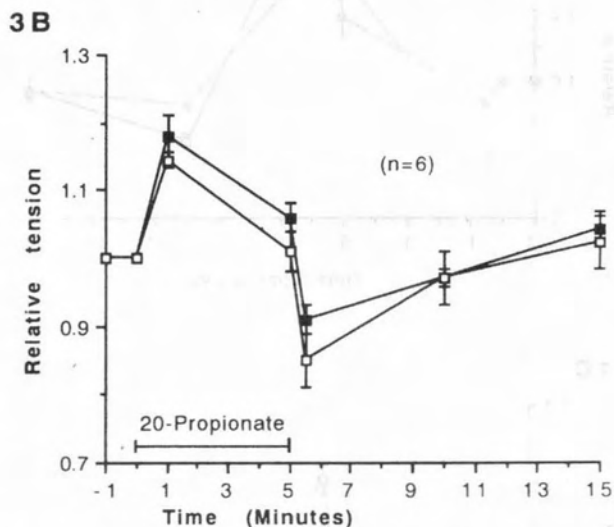


Fig. 3 : Response of the portal veins of SHR (open squares) and their controls of WKY (solid squares) to 5 min pulses of 20 mM sodium propionate. A: preparations are activated with 1 μM NE. B: non-activated preparations.

DISCUSSION

The results show clearly that the portal veins of SHR and WKY dilate to intracellular alkalinity produced by NH₄Cl and trimethylamine application, and they constrict to intracellular acidity induced by washout of NH₄Cl and trimethylamine and application of sodium propionate. This is in general agreement with previous results on the portal vein of Wistar rats, cats and rabbits (14). But what seems to be contradictory results have been reported recently; NH₄Cl application produced constriction (increase in tension) of the nonactivated Wistar rat portal vein preparations which disappeared on washout of the salt (11). These constrictions obtained by NH₄Cl application are most probably due to membrane depolarization effect of the K⁺

like cation NH_4^+ rather than intracellular pH effect (14, 15). The qualitative response of the portal veins of SHR and WKY to variations in intracellular pH is independent of the mode of activation. This supports previous proposals that intracellular pH effect is mediated through direct interaction between intracellular protons ($[\text{H}^+]_i$) and intracellular calcium ions ($[\text{Ca}^{2+}]_i$) (10, 13, 14, 16, 17). Hence a rise in $[\text{H}^+]_i$ would produce a rise in $[\text{Ca}^{2+}]_i$ and subsequent constriction of the vessel. If this relation applies to human vessels then a lower intracellular pH in the vascular smooth muscle of the resistance vessels of hypertensive patients when compared to normotensive individuals may partly explain the hypertension phenomena.

Recovery from acid load in vascular smooth muscle is mainly carried out by Na^+/H^+ exchanger (5, 6). In the present study the recovery from acidic load induced by washout of NH_4Cl or trimethylamine has been slower in SHR portal vein preparations than in WKY ones. The most probable explanation for this is a slower Na^+/H^+ exchanger in SHR portal vein preparations; but a slower activity of $\text{Na}^+/\text{Ca}^{2+}$ can not be excluded from the results in this study. In fact it has

been reported that $\text{Na}^+/\text{Ca}^{2+}$ exchanger is less active in SHR rat tail arterial preparations than in WKY ones (18). However, $\text{Na}^+/\text{Ca}^{2+}$ exchanger plays a minor role in the recovery from NH_4Cl washout constriction (13).

In conclusion, SHR and WKY portal vein preparations dilate to intracellular alkalinity and constrict to intracellular acidity. The recovery from acidic load is slower for the SHR preparations. This is most probably due to a lower activity of the Na^+/H^+ exchanger in SHR than in WKY preparations. Conceivably, a lower intracellular pH in the vascular smooth muscle of the resistance vessels of hypertensive patients, as compared to normotensive individuals, may partly account for the hypertensive phenomena.

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