Changes with hypertension and maturation in the response of stomach fundus to acetylcholine

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Summary. The purpose of these experiments was to compare the contractile response to ACh of stomach fundal strips from hypertensive (SHR) and normotensive (WKY) rats during the development of hypertension. The results indicate that the reactivity to ACh is the same in fundal strips from young SHR and WKY rats; however, with maturation strips from WKY rats undergo a reduction in responsiveness which does not occur in the SHR. Therefore, strips from older SHR rats are more reactive to ACh than are those from age matched WKY rats.

Altman et al.[2] recently observed an increase in responsiveness of stomach fundal strips from SHR rats to Ba, Sr and Ca ions and suggested that a change in the regulation of intracellular Ca may have occurred. This conclusion was substantiated by the work of Kwan et al.[3] in which a lower ^{45}Ca accumulation was observed in microsomal fractions prepared from stomach fundus of SHR rats. The purposes of the present experiments were to extend the observations of Altman et al. to other agonists, and to determine whether the contractile characteristics of fundal strips changed during the development of genetic hypertension.

Materials and methods. Male SHR and WKY rats were obtained from Taconic Farms at 2 different ages: 6–7 weeks old (initial stage of hypertension), and 12–20 weeks old (established hypertension). The systolic blood pressure of the young rats was obtained by direct measurement of carotid pressure during Brevital anesthesia on the day of the experiment. The systolic blood pressure of the older rats was obtained by the indirect tail cuff method[4]. In the case of the older rats, the pressures were measured on several different days prior to the experiment, to insure that a reproducible value was obtained. The rats were sacrificed by decapitation and several longitudinal muscle strips were taken from the fundus of the stomach of each animal and prepared as described by Vane[5]. Strips from SHR and WKY rats were placed together in a 25-ml muscle bath containing an oxygenated (95% O_2, 5% CO_2) Krebs solution: 124 mM NaCl, 4.6 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgCl_2$, 11 mM glucose, 18 mM $NaHCO_3$, pH 7.3 and maintained at 37 °C. One end of the muscle strip was fastened to a movable glass rod, while the other end was attached to an isometric force transducer (Narcobio) by means of silk suture material. An initial preload of 2.0 g was applied to all strips by moving the glass rod and they were allowed to equilibrate for at least 30 min, during which time, the strip length was adjusted to maintain the preload at 2.0 g. After this equilibration period, the strip length was recorded and cumulative concentration-response curves to acetylcholine (ACh), were obtained as described by Van Rossum and Van Den Brink[6].

At the end of the experiment, the wet weight of the tissue was determined and assuming a tissue density of 1.05 g/cm^3, the cross-sectional area of each strip was calculated (area = wt/(lx D)) and used to normalize the tension response[4]. All reported values are means ± SEM. The statistical significance of differences between means was determined by the Student's t-test with a p-value less than 0.05 as the criterion for significance.

Results. The table lists the body weights, and systolic blood pressures of the rats and the calculated cross-sectional areas of the fundal strips at 2 g resting tension. Within a given age group, the body weights of SHR and WKY rats were not significantly different; however, at both ages, the systolic blood pressure of the SHR rats was significantly greater than that of the WKY rats. The calculated cross-sectional area of the fundal strips from the younger animals was significantly less than that of the older animals; however, at a given age, the cross-sectional area of strips from SHR and WKY rats was not different.

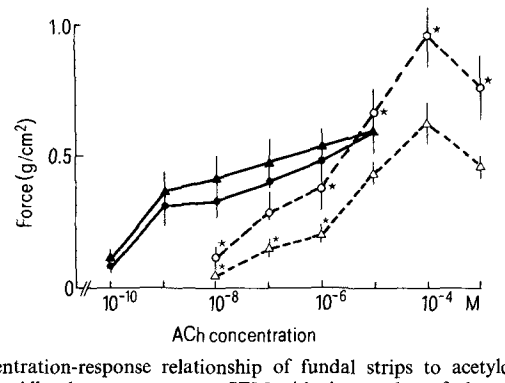
The figure illustrates the concentration-response relationship to acetylcholine of fundal strips from the different groups. Over the concentration range examined, fundal strips from young SHR and WKY rats did not exhibit detectable differences in contractile response; however, in the older age group, fundal strips from SHR rats produced a larger contractile response than did strips from WKY rats at ACh concentrations equal to or greater than 10^{-6} M. Comparing the response of fundal strips from young and old WKY rats the younger age group developed more tension at ACh concentrations of 10^{-6} M or less. This is in contrast to fundal strips from SHR rats in which only at 10^{-8} M ACh did strips from younger animals develop more tension than strips from older animals.

Discussion. Since the hypertension in the SHR rat was produced by selective breeding and all off-spring become hypertensive, the hypertension must be the result of a genetically directed defect. To determine if the contractile properties of all smooth muscle cells are altered, suggesting a genetic alteration of the muscle cell or an effect induced by a circulating humoral substance, the contractile characteristics of venous and nonvascular smooth muscles have been determined[2, 7–10]. Altman et al.[2] have demonstrated a greater contractile response to cal-

Rat and fundal strip characteristics

	6–7-week-old		12–20-week-old	
	WKY	SHR	WKY	SHR
Body weight (g)	118 ± 13 (5)	124 ± 24 (5)	315 ± 9** (11)	309 ± 7** (11)
Systolic blood pressure (mm Hg)	109 ± 5	167 ± 11*	135 ± 4	170 ± 5*
Strip cross-sec area (mm^2)	1.4 ± 0.1 (20)	1.6 ± 0.2 (18)	2.5 ± 0.2** (18)	3.0 ± 0.3** (18)

All values are means ± SEM with number of observations in parentheses. * $p < 0.05$ relative to WKY at same age; ** $p < 0.05$ relative to similar animals in younger groups.



Concentration-response relationship of fundal strips to acetylcholine (ACh). All values are means ± SEM with the number of observations equal to 15–18. Strips from the young animal group are the filled symbols, the old animal group the open symbols, WKY = △ and ▲, SHR = ○ and ●. * $p < 0.05$ relative to WKY of the same age. * $p < 0.05$ relative to young animals of the same type.

cium in stomach fundal strips from adult (24 weeks) SHR rats, when compared to strips from WKY rats. These observations were obtained on strips whose membrane permeability was increased by depolarization with 80 mM KCl and therefore, may have reflected the direct effect of calcium on the contractile system. To determine if the reactivity to membrane receptor mediated contractile agents was altered, the response to ACh was compared in fundal strips from SHR and WKY rats. It was also of interest to determine whether the responsiveness of fundal strips changed during the development of hypertension, since it has been demonstrated that the reactivity of smooth muscle changes with maturation[11-13].

The data presented suggests that the reactivity to ACh of fundal strips from young SHR rats is not altered; however, with maturation an increased reactivity develops. This increased reactivity is due to the fact that strips from SHR rats do not undergo a reduction in reactivity to ACh with maturation that occurs in strips from WKY rats. During maturation of the normotensive rat there is a decrease in reactivity to ACh but this does not occur in the SHR. The experiments of Altman et al.[2] and Kwan et al.[3] suggest that in fundus smooth muscle of mature (> 20 weeks) SHR rats there is a post membrane receptor alteration in Ca^{++} handling which may cause the increased contractile reactivity to divalent cations that they observed. If this is involved in the increased reactivity to ACh of fundal strips from 12–20-week-old SHR rats, then these data suggest that the normal changes in Ca handling that occur with maturation do not occur in the SHR rat and that the fundus may retain its immature Ca^{++} handling properties. Additional experiments need to be performed to test this hypothesis.

In summary these observations indicate that an increase in the contractile effectiveness of ACh occurs in stomach smooth muscle from SHR rats during maturation and substantiate previous experiments suggesting contractility changes in non-vascular smooth muscle with genetic hypertension.

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Accumulation of ^{3}H-dopamine by synaptic vesicles from rat striatum in an impermeant medium

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Summary. The accumulation of ^{3}H-dopamine by synaptic vesicles from rat striatum was significantly stabilized in a membrane impermeant medium. The characteristics of dopamine accumulation by striatal vesicles were quite similar to those reported for dopamine accumulation by a whole brain vesicle preparation in the same medium, and were siginificantly different from the characteristics previously reported for vesicular accumulation of norepinephrine.

We have previously demonstrated that the accumulation of ^{3}H-norepinephrine (NE)[2,3] and ^{3}H-dopamine (DA)[4] by synaptic vesicles from rat whole brain is significantly stabilized in impermeant media, and that the accumulation of NE and DA differ markedly in total levels of accumulation, time course of uptake, kinetic parameters, and sensitivity to ATP and reserpine. To more thoroughly characterize the differences between vesicular accumulation of NE and DA in a whole brain preparation, the accumulation of ^{3}H-DA was examined in vesicles prepared from rat striatum, an area of dense dopaminergic innervation.

Methods. A crude synaptic vesicle fraction was prepared from rat brain striatum as described previously[4]. Sprague-Dawley derived rats of either sex (150–200 g) were sacrificed by decapitation, and the striata quickly dissected and weighed. The tissue was homogenized in 5 volumes of cold buffer (potassium tartrate 103 mM, potassium bicarbonate 3 mM, potassium chloride 1 mM, magnesium chloride 1 mM, potassium phosphate 4 mM, iproniazid phosphate 10 µM, ascorbic acid 10 µM and EDTA 1 µM, pH to 7.5 with KOH) with 6 up-down strokes of a hand-held Duall glass/glass homogenizer. The resulting suspension was sequentially centrifuged at 4 °C (3000 × g, 20 min; 20,000 × g, 30 min; 100,000 × g, 45 min) to afford a crude vesicle pellet (1.58 mg protein per g of wet tissue). The pellet was gently resuspended in 500 µl of cold buffer using slow up-down strokes of a Duall glass-teflon homogenizer.

Uptake studies were performed by adding 250 µl of a resuspended vesicle pellet to 710 µl of cold buffer. The suspension was preincubated for 2 min at 37 °C, at which time 20 µl of K_2ATP was added to give a final concentration of 2 mM. After 2 min 40 µl of ^{3}H-DA (20 µCi) was added to give a final concentration of 10^{-5}M DA in a final volume of 1 ml. At specified intervals 100 µl aliquots were filtered on Whatman GF/A glass fiber filters, and immediately washed with 5 ml of buffer. The tritium content of the filters was determined by liquid scintillation counting (minimum efficiency 30%). All samples were corrected for quenching. Filter blanks were subtracted from experimental points. When employed, additional drugs were added to the vesicles 1 min prior to tracer addition. For kinetic studies, 160 µl aliquots of tissue were incubated as described above. 20 µl aliquots of ^{3}H-DA at various concentra-