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-

tions, and 20 µl aliquots of K_2ATP were added to give a final volume of 200 µl. Uptake was terminated 4 min after addition of tracer.

Protein determinations were done colorimetrically using the biuret reagent. (7-^{3}H) Dopamine (34 Ci/mM) and Atomlight scintillation fluid were purchased from New England Nuclear Corp. Dopamine, dipotassium ATP and FCCP (carbonylcyanide-p-trifluoromethoxyphenylhydrazone) were obtained from Sigma Chemical Company. Reserpine (Serpasil) was obtained from Ciba. Sprague-Dawley derived rats were obtained from Biological Research Labs, Arvada, Colorado.

Results. The time course of ^{3}H-DA accumulation by synaptic vesicles from rat striatum is shown in figure 1. DA accumulation saturated by 18 min at 37 °C and remained stable for periods up to 1 h. Uptake was reduced by approx. 50% in the presence of 10^{-6}M reserpine, and by 90% at 4 °C or in the presence of a proton ionophore FCCP (10^{-6}M). In the absence of ATP, DA accumulation was stable for 12 min, at which time apparent levels of accumulation increased sharply, reflecting a loss of stability of the system. This effect was prevented in the presence of 10^{-6}M reserpine. The total levels of DA accumulation under various conditions are shown in table 1. A 1-way analysis of variance revealed significant main effects for the various uptake conditions ($F[5,58] = 202.135$, $p < 0.001$). A kinetic analysis of DA accumulation by striatal vesicles is shown in figure 2. Lineweaver-Burk analysis revealed an apparent Km of $6.2 \pm 1.5 \times 10^{-5}$ ($r^2 = 0.99$).

Discussion. We have previously demonstrated that a preparation of rat whole brain storage vesicles displays extended biochemical stability in a medium comprised largely of membrane-impermeant anions[2]. This observed extension of biochemical viability was shown to be due to a reduction in osmotic swelling and lysis that occurred when vesicle transmembrane gradients were established in the presence of small, permeant anions. The use of such media has allowed characterization of stable, ATP-dependent vesicular accumulation of (−)-^{3}H-NE[2,3], (−)-^{3}H-glutamic acid[8], and ^{3}H-DA[4].

In the course of initial characterization of vesicular accumulation of ^{3}H-DA, a whole brain vesicle preparation was employed to determine if the characteristics of DA and NE accumulation significantly differed. The accumulation of ^{3}H-DA was indeed significantly stabilized in an impermeant medium, and the characteristics of accumulation were markedly different than those observed for NE uptake[4]. However, several characteristics of DA accumulation raised questions as to the selectivity of uptake. In particular, the observed apparent Km value of 2.1×10^{-5}M was 5- to 10-fold larger than had been observed by other investigators[5-7]. In addition, it was found that vesicular DA accumulation was reduced only 25% in the absence of ATP, and 40% in the presence of 10^{-6}M reserpine, although the uptake was quite sensitive to temperature and the proton ionophore FCCP. These results suggested the possibility of non-selective incorporation of DA into non-dopaminergic vesicles. To help clarify this situation, the present study was undertaken to characterize DA accumulation by vesicles from rat striatum, an area of rich dopaminergic innervation. The time course of ^{3}H-DA accumulation by striatal vesicles stabilizes at 18 min, and remains constant over periods of 30–60 min. This time course is quite similar to that obtained with a whole brain vesicle preparation[4]. Uptake of 37 °C was reduced approx. 50% in the presence of 10^{-6}M reserpine, again quite similar to the reserpine sensitivity observed with whole brain vesicles[4]. The apparent Km value of 6.2×10^{-5}M for ^{3}H-DA accumulation by striatal vesicles is quite close to the value of 2.1×10^{-5} observed for DA accumulation in whole brain vesicles.

At 4 °C, or in the presence of 10^{-6}M FCCP, a proton-translocating ionophore, DA accumulation is reduced by approx. 90%, as would be expected for a carrier-mediated, gradient-linked transport. Total levels of ^{3}H-DA accumulation are 7-fold higher than the levels observed in a whole brain vesicle preparation, which would be expected for a brain area of dense dopaminergic innervation.

The only marked difference between the present study with striatal vesicles and the previous study[4] with whole brain vesicles is the pronounced lack of stability of the striatal preparation in the absence of ATP. Vesicular DA accumulation is reduced by approx. 50% in the absence of ATP for 12 min, at which time apparent accumulation rose sharply to nearly twice control levels. It is not clear what causes this lack of stability. However, the presence of 10^{-5}M DA in the absence of ATP is a non-physiological situation which could give rise to non-specific exchange of DA with other endogenous substances. The possibility of vesicle aggregation or swelling, with non-specific

^{3}H-DA accumulation (pm/mg protein) by synaptic vesicles from rat striatum**

Control (ATP, 37°)	278.0 ± 15.8
+ ATP + Reserpine (10^{-6} M)	143.5 ± 14.7*∫
− ATP + Reserpine (10^{-6} M)	122.2 ± 9.6*∫
+ ATP 4 °C	25.8 ± 3.8*†
+ ATP + FCCP (10^{-6} M)	35.0 ± 4.4*†

* Significantly different from Control ($F[5, 58] = 202.135$, $p < 0.001$); ∫ Not significantly different; † Not significantly different; ** Vesicles were incubated with ^{3}H-DA (10^{-5} M) for 15 min under the specified conditions.

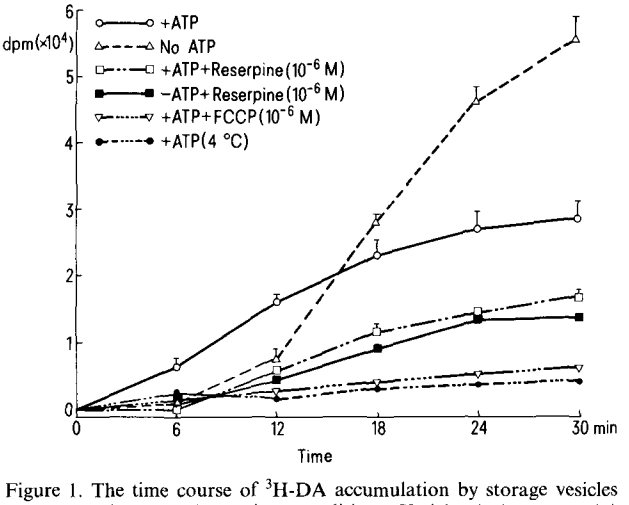

Figure 1. The time course of ^{3}H-DA accumulation by storage vesicles from rat striatum under various conditions. Vesicles (1.5 mg protein) were incubated at 37 °C with ^{3}H-DA (10^{-5}M) and K_2ATP (2mM) in 2 ml of buffer. Aliquots (100 µl) were filtered and washed with 5 ml of warm buffer. Mean ± SEM of 3–6 determinations.

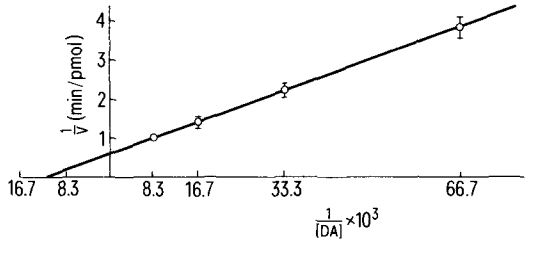

Figure 2. Lineweaver-Burk plot for the uptake of ^{3}H-DA by synaptic vesicles from rat striatum. Vesicles were incubated with various concentrations ^{3}H-DA and K_2ATP (2 mM) in a volume of 200 µl (0.1 mg tissue). Uptake was terminated by filtration after 4 min. Mean ± SEM for 3 determinations in duplicate.

occulsion of DA cannot be excluded. Interestingly, the phenomenon is prevented in the presence of 10^{-6}M reserpine (fig. 1). HPLC analysis of endogenous catecholamines in the vesicle pellet (electro-chemical detection) reveals values of 90.0 ± 12.8 pM/mg protein of DA, and 39.8 ± 12.8 pM/mg protein of NE (n = 6), and reflects substantial loss of endogenous DA during vesicle isolation. Exchange with these remaining endogenous substances could not lead to the DA levels observed after 12 min in the absence of ATP.

We have recently shown that the filtration assay employed in these studies captures approx. 30% of the vesicle preparation filtered[9]. Thus, levels of ^{3}H-DA accumulation in vesicles from rat striatum at 37 °C would be in the range of 900 pmol/mg of vesicle protein, a value over 20 times that of endogenous NE in the crude vesicle pellet, and more in line with relative DA and NE levels found in rat striatum[10]. In view of the similarity of characteristics of vesicular DA accumulation in whole brain vesicles[4] and the present study with striatal vesicles, and in view of the greatly elevated level of DA accumulation in striatal vesicles (278 pM/mg protein versus 41.8 pM/mg protein in whole brain vesicles), the data suggest that vesicular DA accumulation by a whole brain vesicle preparation in an impermeant medium may reflect DA uptake by dopaminergic vesicles.

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Effects of sympathetic stimulation on ventricular refractory periods in cats with acute coronary artery ligation[1]

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Summary. Ventricular refractory periods shorten in the ischemic area following acute coronary artery ligation. Subsequent bilateral sympathetic nerve stimulation reduces disparity in refractory periods across normal, border (peri-ischemic) and ischemic areas.

A higher incidence of cardiac arrhythmias occurs during coronary artery occlusion and sympathetic stimulation than during coronary occlusion alone[2-4]. Increased dispersion of ventricular refractory periods induced by sympathetic activation[5] has been suggested as a mechanism for the enhanced arrhythmogenesis. Recently, Burgess and Haws[6] reported the first data on the effects of sympathetic stimulation on ventricular refractoriness in the acutely ischemic canine heart and found that heightened sympathetic tone reduces inequalities in refractoriness by prolonging refractory periods in ischemic sites while shortening them in non-ischemic sites. The present results confirm their finding that sympathetic activity reduces disparity in refractory periods in the acutely ischemic heart, although the effects of sympathetic stimulation on ischemic tissue noted by us were opposite to those described by Burgess and Haws[6].

Materials and methods. Experiments were carried out in adult cats (2.0–4.7 kg) maintained under sodium pentobarbital (30 mg/kg, i.p. and supplemental doses) anesthesia and ventilated. Blood gasses and temperature were maintained in the normal range. In each cat, the sinus node was crushed and the right atrium and posterior surface of the left ventricular free wall (near the base) were paced with 2-msec pulses delivered at 1.5 times threshold voltage. Pacing cycle length was held constant for each cat and ranged from 301–350 msec for the cats studied. Myocardial ischemia identified by discrete epicardial cyanosis was induced by single-stage ligation of distal tributaries of the left anterior and circumflex coronary arteries as described previously[7,8] and allowed to evolve for 1 h. The same procedure was followed for sham-operated control cats except that non-occlusive ties were used. Epicardial bipolar electrodes were used to deliver extra-stimuli and to record surface electrograms in 3 areas on the left ventricle in each ligated heart: 1) center of the ischemia (ischemic area), 2) area bordering the ischemia (peri-ischemic or border area), and 3) normal tissue proximal to the ischemia (normal area)[7,9]. Epicardial areas studied in sham-operated control hearts corresponded to the ischemic, border and normal areas in ligated hearts and are referred to as distal, central and proximal control areas, respectively. To measure refractory periods, a train of 10 pacing stimuli were delivered to the right atrium (S_1) and left ventricle (S_2), with an S_1–S_2 interval (A–V delay) of 60–90 msec. S_2 was followed by a late diastolic extrastimulus (S_3) delivered to 1 of the 3 epicardial areas. The S_2–S_3 coupling interval then was shortened in 2 msec decrements until failure to capture occurred. The S_2–S_3 interval then was increased by 10 msec, and shortening was repeated until failure to capture occurred a second time. The refractory period was defined as the longest S_2–S_3 interval at which S_3 failed to capture the ventricle. Extrastimuli were 2-msec pulses, delivered at 2 or 15 times late diastolic threshold. Bilateral sectioning of the vagi, and decentralization of the stellate ganglia (only attachments to the right and left anterior and posterior ansae subclaviae were left intact) was carried out 1 h after coronary ligation or sham-operation. Bilateral sympathetic nerve stimulation was accomplished by delivering 4-msec pulses at 15 Hz and 2–10 mA to