droxylase is regulated by the estrous cycle and gonadotropins in a manner similar to that of DMBA hydroxylase.

Our results indicate that ovarian DMBA hydroxylase is regulated by the pituitary and that EH, DT, and GST are not. To our knowledge, this is the first time that DMBA hydroxylase has been shown to undergo short-term cyclic changes controlled by endogenous factors under physiological conditions. This finding may cast light on the role of PAH-metabolizing hydroxylases in endocrine organs. The estrous cycle dependence of DMBA hydroxylase suggests that the normal substrate is a steroid, a possibility supported by the sensitivity of this enzyme to 17-hydroxylase inhibitors (20). Physiologically, the relation between the level of gonadotropins secreted by the pituitary and DMBA hydroxylase activity indicates that the rate of ovarian cancer initiation increases under conditions of increased secretion of gonadotropins. This is supported by the finding that excessive stimulation of grafted rat ovaries by gonadotropins, in the presence or the absence of added xenobiotics, leads to an increase in ovarian cancer (21, 22). Thus the action of gonadotropins on DMBA hydroxylase may be one of several hormone-dependent effects, including those at the level of gene expression and DNA replication. It is interesting to note the high incidence of ovarian cancer following menopause (3, 4) and the low incidence in women taking contraceptive pills (23), conditions that lead to increased and decreased levels of gonadotropins, respectively.

Ovarian benz[a]pyrene hydroxylase is induced by PAH's (14). The relation between the cellular mechanism in pituitary hormone regulation and PAH regulation of DMBA hydroxylase and benz-[a]pyrene hydroxylase remains to be elucidated, as does the clinical significance of the pituitary regulation of PAH-metabolizing hydroxylases.

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Tension Transients in Single Isolated Smooth Muscle Cells

Abstract. Tension transients were recorded in a single smooth muscle cell. The transient contains a linear elastic response and a biphasic recovery that appear to originate from the cross-bridges. A comparison of transients in smooth and fast skeletal muscle fibers suggests that the cross-bridge in smooth muscle is more compliant than the cross-bridge in striated muscle and that transitions between several cross-bridge states occur more slowly in smooth muscle than in striated muscle.

The cyclic interaction of myosin cross-bridges with actin filaments in smooth muscle is believed to be responsible for force generation or active cell shortening (1). Although the contractile mechanism in smooth muscle is similar to that in striated muscle, the contraction of smooth muscle has several distinctive features. The velocity of shortening and rate of the actomyosin adenosine triphosphatase are much slower in smooth muscle than in striated muscle. In addition, maximum force production in smooth muscle is comparable to that in striated muscle but requires much less myosin. These differences may be explained in part by differences in the cross-bridge cycle. As several steps of the cycle are believed to be sensitive to the position of the cross-bridge relative to its actin binding site, rates of transition between cross-bridge states (that is, cross-bridge kinetics) may be tested by application of small force or small length perturbations (2). The multiphasic tension transients observed in frog skeletal muscle in response to small and rapid changes in length are believed to reflect the kinetics of various steps in the cross-bridge cycle. Similar studies have been performed on several smooth muscles (3, 4), but marked differences in the tension transients were observed in different tissues. Furthermore, none of the tension transients observed in smooth muscle

were similar in form to those observed in single striated muscle fibers. Although the differences in the tension transients of smooth and striated muscle might reflect real differences in their contractile machinery, they might also reflect differences in preparation—for example, a single striated muscle fiber compared with a more complex multicellular segment of smooth muscle.

We investigated the force-generating mechanism in smooth muscle by observing the response of single isolated smooth muscle cells (SMC) to rapid changes in length during isometric force production. For this purpose, we developed the means to measure force from such a small cell, as well as the techniques required to attach the cell to a force-measuring device (5). Single SMC were obtained by enzymatic disaggregation of the stomach muscularis of the toad *Bufo marinus*. These cells are typically 150 μm long and 6 μm wide and have no tendinous connections. A short portion of each end of a single cell was tied by micromanipulation around special probes. The probes were connected to a length driver for precise control of cell length and to a newly designed force transducer for measurement of the force response. The new transducer, which has greater resolution (1 μg) and a higher natural frequency (425 Hz) than those used previously for recording isometric

force (5), was developed specifically for the much smaller (~ 10 μg) and faster (< 2 msec) tension fluctuations expected after a rapid change in the length of a single SMC.

Three general features were apparent when this method was used to record tension transients in a single SMC in response to either a rapid release or stretch (Fig. 1, C and D): (i) an immediate tension change that coincided with the applied length step; (ii) a rapid partial recovery of tension that occurred during the length step; and (iii) a further recovery of tension, on completion of the length step, that was resolvable into two components and resulted in virtually complete recovery in 1 second. The possibility that the cell ends were damaged in the knotting procedure was ruled out by making strobe photographs of the distribution of marker beads on the surface of the cell after the length step was completed and comparing them with similar photographs made before the length step was begun (Fig. 1, A and B). They revealed that the length change was uniformly distributed over the cell. Thus, the tension transients appear to reflect the response of the cell as a whole, and the knotted portions do not appear to represent areas of high compliance that might seriously distort the tension transients.

In a single striated muscle fiber, the relation between length and force during the length step is believed to reflect the properties of a linear elastic element within the cross-bridge (6). When the length-force relation was examined during releases or stretches in a single SMC at the peak of force production (Fig. 2, A and C), force was not linearly related to length as it is in striated muscle. Similar, but often more exaggerated, nonlinear relations of length to force have been observed in smooth muscle tissue studies (3, 4). The nonlinear length-force relation in the isolated cell may reflect the fiber's true elastic properties or, alternatively, may be the result of superposition of recovery processes on the elastic response. Evidence for the latter is provided by comparison of the length-force relation in the same fiber during releases at two different speeds (Fig. 2C). The deviation from linearity in the slower release (25 msec) is minimized when a faster release (5 msec) is applied, since the faster release occurs at a rate comparable to the rate of recovery. These results indicate that recovery takes place during the length step and may account at least in part for the nonlinear length-force relation in the single SMC. Similar recoveries may also

play a role in the nonlinear length-force relation in intact tissue. However, the presence of a complex connective tissue matrix within the tissue may also contribute to the observed nonlinearity of the length-force relation. This suggestion is supported by the observations of Mulvany and Warshaw (7) that at least 20 percent of the series elasticity in a small artery preparation is in the connections between SMC. Our isolated cells are free from this connective tissue matrix and thus the nonlinearity of the length-force relation cannot be attributed to an extracellular elasticity.

The true underlying elastic response of the single cell can be characterized only if tension recovery during the length step

is significantly reduced. This can be accomplished either by applying faster length steps or by reducing the cell temperature sufficiently to slow the recovery processes. Although these experimental procedures are straightforward, the technical difficulties of implementing them on the single cell have prevented us from using them to date. Instead we have approached the problem mathematically (6) by assuming that the observed response during the length change reflects an elastic response and that the recovery during the length change is identical to the recovery observed after completion of the length change (Fig. 1, E and F). The observed nonlinear responses during a length change of 0.75 percent of the
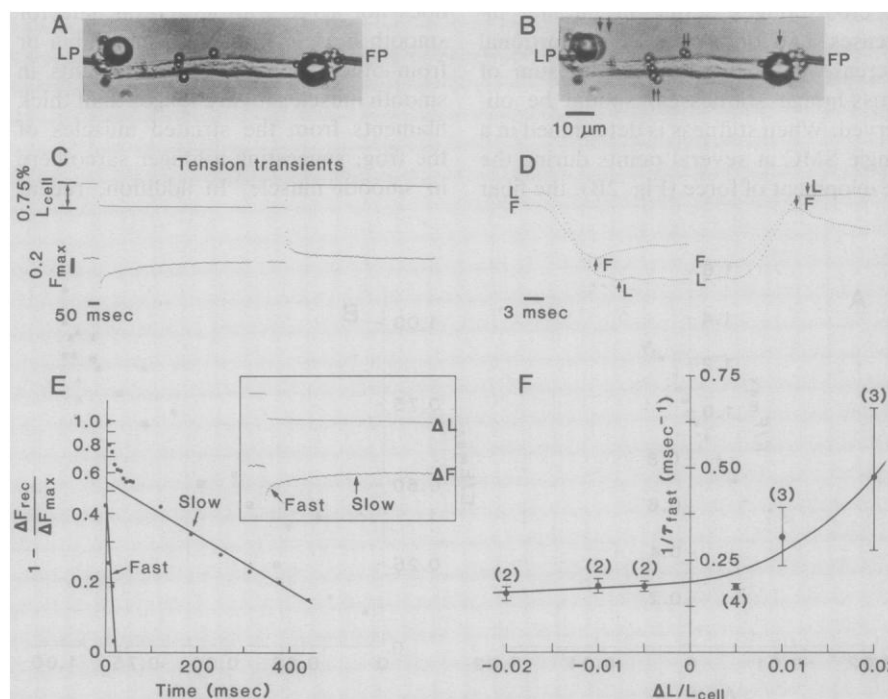

Fig. 1. (A) Single smooth muscle cell tied between two microprobes. A single cell was attached by anionic exchange resin beads to two microprobes, with knots tied by micromanipulation (5). The microprobes, *FP* and *LP*, were connected to a force transducer and piezoelectric displacement device (maximum response, ± 2.5 percent of L_{cell} in 1.0 msec), respectively. The position of the displacement device was detected by an eddy current sensor (frequency response, 5 kHz; resolution, 0.03 μm). Cells were maximally activated by transcellular electrical stimulation through platinum paddles. Tiny resin beads on the cell surface act as length markers. (B) Double-exposure flash photograph showing changes in bead spacing (arrows) confirmed that the displacement sensor provided an accurate electronic record of the length step. The length step was distributed uniformly throughout the cell. (C) A typical pair of tension transients in response to a release and stretch of 0.75 percent of L_{cell} completed in 5 msec at the peak of contraction. Tension records are computer traces after digital correction for the force transducer's mechanical properties (6). (D) Tension transients as in (C) at a shorter time scale. The maximum force change occurred before completion of the step (arrows) and reversed direction, indicative of a recovery in force during the length step itself. (L_{cell}, 175 μm; F_{max}, 214 μg). (E) The tension recovery (see inset) after completion of the length step is described by a fast and slow exponential process. To demonstrate this fact, tension data are plotted semilogarithmically as one minus fractional recovery versus time. The fast component was obtained by subtracting the line describing the slow component from the observed data. The usual method for determining the magnitude and rate constant of the two exponential processes utilized a nonlinear regression by least-squares analysis. (F) The amplitude of the length step versus the rate constant ($1/\tau_{fast}$) of the fast recovery process. Data from three cells are shown with the curve of the best fit [$1/\tau_{fast} = 0.19 + 4.43 \ \Delta L/L_{cell} + 323.33 \ (\Delta L/L_{cell})^2 + 8627.45 \ (\Delta L/L_{cell})^3$; $r^2 = .95$] drawn through the means and standard errors. The numbers in parentheses indicate the total number of trials obtained from these cells for a given amplitude step.

cell length (L_{cell}) are accounted for if we assume a linear fiber elasticity 77 times the ratio of the maximum force (F_{max}) to L_{cell} on which is superimposed recovery processes identical to those that follow the completion of the length step. These results also indicate that a good estimate of the true elastic response may be obtained from releases of less than 0.5 percent of L_{cell} completed in under 5.0 msec.

In striated muscle, where the true elastic response has been probed by the use of very rapid length steps, the slope of the length-force curve during the length change is believed to be a measure of the cross-bridge stiffness—that is, the reciprocal of the compliance (6). If this is true for smooth muscle, then as the number of cross-bridges acting in parallel increases and force rises, a proportional increase in fiber stiffness (the sum of cross-bridge stiffnesses) should be observed. When stiffness is determined in a single SMC at several points during the development of force (Fig. 2B), the fiber

stiffness varies in proportion to force. This result supports the view that the elastic responses recorded originate within the cross-bridges.

Since the elastic response in the single SMC appears to reflect the mechanical properties of the cross-bridges, we can compare these properties to those in striated muscle. By extrapolating the linear relation for the elastic response of the cell (Fig. 2C), we find that a release of 1.3 percent of L_{cell} should cause the force to drop to zero. This represents a considerably larger release than the 0.5 percent per half-sarcomere in fast skeletal muscle (6). If we assume that a mechanically similar cross-bridge exists in smooth muscle, then the effective half-sarcomere should be 0.4 μm. This value does not agree with structural data for smooth muscle from these amphibia or from other species. Thick filaments in smooth muscles (8) are longer than thick filaments from the striated muscles of the frog, suggesting a longer sarcomere in smooth muscle. In addition, recent

immunocytochemical studies reveal that in single SMC, discretely stained cytoplasmic bodies demarcate the borders of adjacent sarcomere-like units having a mean length of 2.2 μm in relaxed cells (9). Thus, mechanical data coupled with structural data for smooth muscle suggest that differences in the elastic properties of smooth and skeletal muscle fibers may be explained by some difference in the cross-bridges, whose properties are being probed.

The tension recovery on completion of the length step can be adequately described by two exponential processes (Fig. 1E) having the following time constants: τ_{fast}, 5 to 20 msec, and τ_{slow}, 50 to 300 msec. Because the two phases of tension recovery were not separated at 20°C by a phase involving reversal of the recovery, as is the case in striated muscle, the two phases were defined by mathematical analysis rather than by direct inspection of the records. We believe that the recoveries do reflect cross-bridge activity since this recovery is lost in rigor-like cells that can no longer shorten after prolonged activation (> 2 minutes), although both maximum force and stiffness remain elevated. The two exponential recoveries in the single cells are similar in form to that observed in the rabbit urinary bladder (4). However, both the fast and slow recovery phases in the single cell occur at rates two or three times faster than the recovery in the tissue after correction for differences in temperature. The difference in recovery rates cannot be attributed to differences in the shortening velocities of the bladder preparation and the single cell since the maximum velocity of shortening in the tissue at 20°C would be twice that of the single cell (10). We suggest that the slower tension recoveries in the tissue as compared to the single cell may be due to the presence of a significant series elasticity through which the cross-bridge response must be transmitted. In addition, heterogeneity in the response of individual cells within the tissue may further increase the recovery time in the tissue relative to that in the SMC.

The rates of both fast and slow phases of tension recovery in a single SMC at 20°C are at least an order of magnitude slower than the rate in fast striated muscle at 4°C (6). This is in accord with recent energetic and biochemical studies which suggest that the overall actomyosin cycle as well as a number of specific steps in the cycle are slower in smooth muscle than in striated muscle (11). If similar processes determine the fast phase of tension recovery in smooth
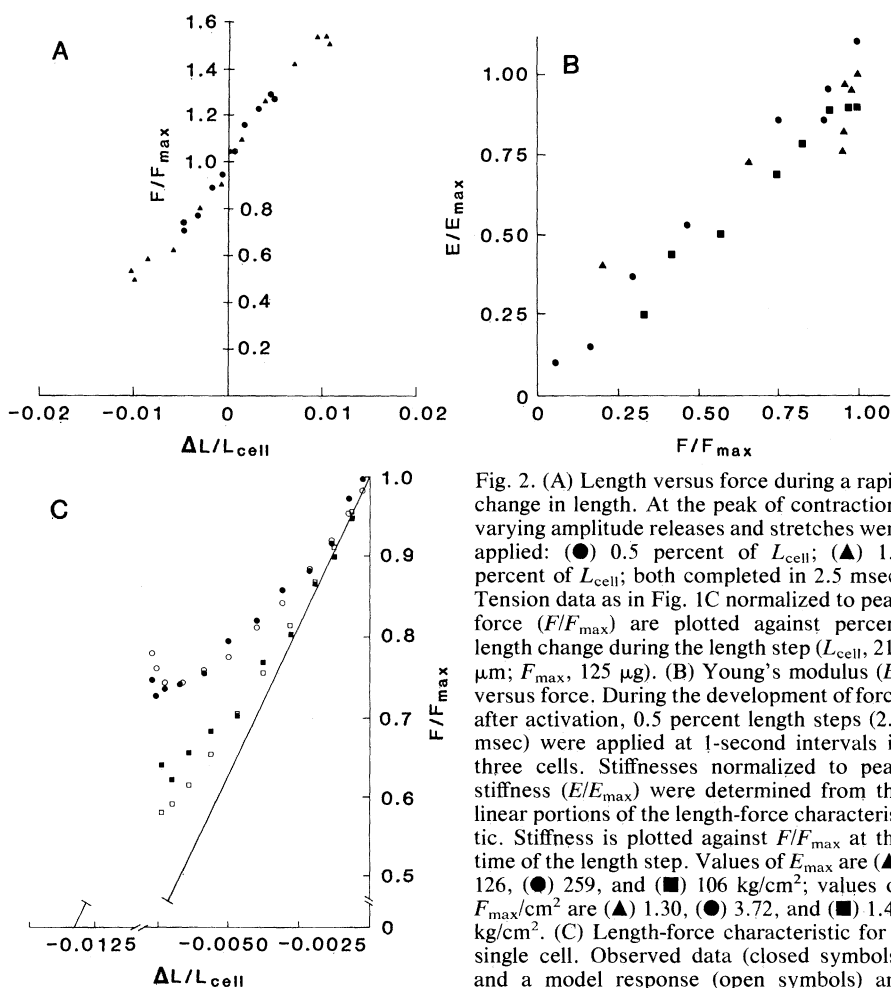

Fig. 2. (A) Length versus force during a rapid change in length. At the peak of contraction, varying amplitude releases and stretches were applied: (●) 0.5 percent of L_{cell}; (▲) 1.0 percent of L_{cell}; both completed in 2.5 msec. Tension data as in Fig. 1C normalized to peak force (F/F_{max}) are plotted against percent length change during the length step (L_{cell}, 217 μm; F_{max}, 125 μg). (B) Young's modulus (E) versus force. During the development of force after activation, 0.5 percent length steps (2.0 msec) were applied at 1-second intervals in three cells. Stiffnesses normalized to peak stiffness (E/E_{max}) were determined from the linear portions of the length-force characteristic. Stiffness is plotted against F/F_{max} at the time of the length step. Values of E_{max} are (▲) 126, (●) 259, and (■) 106 kg/cm²; values of F_{max}/cm² are (▲) 1.30, (●) 3.72, and (■) 1.45 kg/cm². (C) Length-force characteristic for a single cell. Observed data (closed symbols) and a model response (open symbols) are plotted for releases of varying speeds: (■ and □) 5 msec, (● and ○) 25 msec. Model response was determined as described in text. The linear elastic (solid line) response is extrapolated to zero force with an intersection of 1.3 percent of L_{cell}. The mean ± standard error for nine cells was 1.6 ± 0.2 percent.

muscle fibers and fast skeletal muscle fibers, then the differences observed in various aspects of this tension recovery may reflect important differences in the cross-bridge mechanism in the two muscle types. To determine whether similar processes underlie the fast phase for the two muscle types, stiffness in the single SMC was assessed with sinusoidal (250 Hz) length perturbations of small amplitude during the fast phase of tension recovery. In single SMC, as in fast skeletal muscle fibers (12), stiffness changed less during the fast phase of tension recovery than might be expected from the known relationship of steady-state force and stiffness. This suggests that similar processes may underlie the fast phases of tension recovery in smooth muscle and fast skeletal muscle. In fast skeletal muscle, this phase of tension recovery has been interpreted as reflecting transitions of a portion of the attached cross-bridge population from an attached low-force state to a high-force state. Therefore, this transition may be much slower in smooth muscle as predicted by our data.

During the fast phase, the rate of recovery varied little as the magnitude of release was increased, but increased as the magnitude of the stretch was increased (Fig. 1F). This length dependence of the rate constant for rapid tension recovery in single SMC is opposite to that observed in fast skeletal muscle fibers; in the latter, the rate constant increases in magnitude from the largest stretches to the largest releases (6). Tension transients in slow skeletal muscle fibers of the tortoise (13) show a length dependence of the rate constant during the fast phase of tension recovery similar to that in our SMC. Tortoise skeletal muscle is similar in structure to fast skeletal muscle (14) but resembles smooth muscle in the high economy of its contraction (15). The similarity in the length dependence of the rate constant in the slow skeletal muscle of the tortoise and in SMC may indicate that differences in the inherent length dependence of steps in the cross-bridge cycle may be a common feature of slow economical muscles.

Our results indicate that information about the cross-bridge mechanism can be obtained directly at the cellular level. The tension transients observed in a single SMC suggest that the elasticity of cross-bridges in smooth muscle is linear and more compliant than the elasticity of cross-bridges in fast skeletal muscle and that transitions between attached cross-bridge states occur at least an order of

magnitude more slowly in smooth muscle. Further analysis of the tension transient in single smooth muscle cells should aid our understanding of the characteristic ability of smooth muscle to produce forces equivalent to those of striated muscle while using much less energy and having considerably less myosin.

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Overproduction and Elimination of Retinal Axons in the Fetal Rhesus Monkey

Abstract. *Quantitative electron microscopic analysis reveals 2.85 million retinal axons in fetal rhesus monkeys—a number that is more than twice the 1.2 million present in the adult. More than 1 million supernumerary optic axons are eliminated before birth, simultaneously with the segregation of inputs from the two eyes into separate layers of the lateral geniculate nucleus. Selective elimination of optic axons may not only play a role in the segregation of binocular visual connections but, secondarily, may establish the ratio of crossed and uncrossed retinogeniculate projections.*

In all vertebrate species the eyes are connected with the brain by the optic nerve, which contains axons that originate from retinal ganglion cells and terminate in several diencephalic and mesencephalic structures. As a rule, the two optic nerves converge and cross the midline in the form of an X-shaped optic chiasm before reaching the brain. In most mammals a certain number of fibers do not cross the midline but project instead to the ipsilateral side of the brain. The proportion of crossed to uncrossed fibers generally correlates with the degree of overlap between the two visual fields (1). Thus, in rodents with laterally
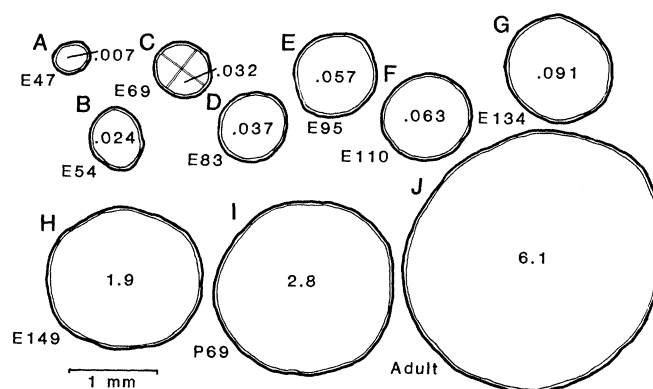

Fig. 1. Cross-sectional areas of 11 optic nerves in a series of monkeys of ascending ages. The thin line marks the nerve boundary, and the thick one, the meningeal membrane. The two diagonal stripes on nerve C illustrate orientations of the electron microscopic photomontages used for quantitative analysis (for example, Fig. 2A).