Contracture and Catecholamines in Mammalian Myocardium

Abstract. *Contractures induced by KCl are produced in cat ventricular muscle after depletion of catecholamine stores by previous treatment with reserpine or by selective blocking of adrenergic receptor sites with propranalol. Contractures are reproducible for 2 to 3 hours and are markedly depressed by the addition of epinephrine. The relaxation of contracture induced by epinephrine parallels the effect of this compound of shortening the duration of the normal twitch. The failure of the normal mammalian myocardium to maintain tension in solutions of high KCl concentration appears to be related to the endogenous amounts of catecholamines in the tissue.*

Maintained tension (contracture) is readily produced in frog ventricle with exposure to high KCl concentrations, but the response is often absent or less marked in the mammalian (rat, rabbit, cat, and sheep) ventricle. I report conditions where contractures are produced in mammalian heart and discuss the significance of the difficulty of eliciting contractures from normal myocardium.

Papillary muscles or ventricular trabeculae, or both, were excised from healthy male cats as well as cats treated with reserpine (1 mg of reserpine per kilogram of body weight, intraperitoneal for 3 days). Isometric tension was measured with a capacitance transducer (*1*). Muscles were equilibrated for 1 hour in normal Tyrode's solution (concentrations in millimoles: NaCl, 137; KCl, 2.7; $MgCl_2$, 0.69; $CaCl_2$, 1.8; NaH_2PO_4, 0.4; $NaHCO_3$, 11.9; and glucose, 5 to 7; buffered to *p*H 7.4) before the start of the experimental procedure. In some cases $10^{-6}M$ propranalol (*2*) was added to the perfusate during the equilibration period. All experiments were carried out at 26°C and at variable frequencies of stimulation (5 to 20 beat/min).

Exposing a normal muscle to 100 m*M* KCl Tyrode's solution (KCl substituted for NaCl) produced contractures ranging in amplitude from 1 to 10 percent of the contraction force. The muscles (from animals treated with reserpine) produced maintained tensions ranging from 70 to 110 percent of the twitch tension for the duration of exposure to KCl (2 to 4 minutes). Similar results were obtained when muscles equilibrated in Tyrode's solution containing $10^{-6}M$ propranalol were used. Figure 1a shows the contractile response obtained from a normal heart. The contracture response is small compared to the twitch; when Tyrode's solution is again added to the muscle, the muscle returns to the control state. Figure 1b shows the response of a muscle treated with reserpine to solutions of high KCl concentrations. The muscle produces marked tension for the duration of depolarization. Such contractures are reproducible for at least an hour; thereafter they start to decrease in amplitude. Initially the twitch in the muscles treated with reserpine is usually very long (2.5 to 3 seconds at 26°C). With repeated exposures to the solution of high KCl concentration the twitch shortens markedly, the relaxation phase in particular. The shortening of the twitch is often accompanied by a decrease in the amplitude of the KCl contracture.

Figure 1b also shows the effect of epinephrine (1 μg/ml) administered at the peak of the contracture response. The muscle contracture partially relaxes and acquires a new steady state. Subsequent exposures of the muscle to KCl solutions containing epinephrine produce little if any contracture tension in spite of a 50 to 100 percent increase in twitch tension and a dramatic shortening of the twitch. Exposure of the muscles treated with propranalol to KCl solutions produced marked contractures (70 to 110 percent of twitch height) for the duration of KCl exposure (Fig. 2). Contractures of muscles treated with propranalol were reproducible for 2 to 3 hours. Unlike preparations treated with reserpine, the duration of the twitch in muscles treated with propranalol remained constant with repeated exposures to KCl.

Since catecholamines prevent or actively relax the contracture tension in myocardium (*3*, *4*), it may be argued that the difficulty of producing contractures in the mammalian myocardium is based on the endogenous release of catecholamines liberated from the nerve endings in the tissue during KCl exposure. Such a hypothesis would require that: (i) Catecholamines should prevent or diminish contractures. (ii) Agents specifically blocking catecholamine receptor-sites in myocardium (for example, propranalol), should enhance contractures produced by KCl. (iii) Agents depleting catecholamine stores in nerve endings (for example, reserpine), should amplify the development of KCl contractures. (iv) Potassium chloride should release catecholamines from the tissue.

The experimental data support the first three requisites. That there is release of catecholamines from the myocardium in the presence of increased KCl concentrations (iv) has been demonstrated in vitro and in vivo. Haeusler *et al.* (*5*) show a progressive increase in the amount of catecholamines released from the heart as the KCl concentrations were increased from 50 to 160 mmole.

The progressive decrease in contracture response to repeated exposure of KCl in muscles treated with reserpine accompanied by shortening of the

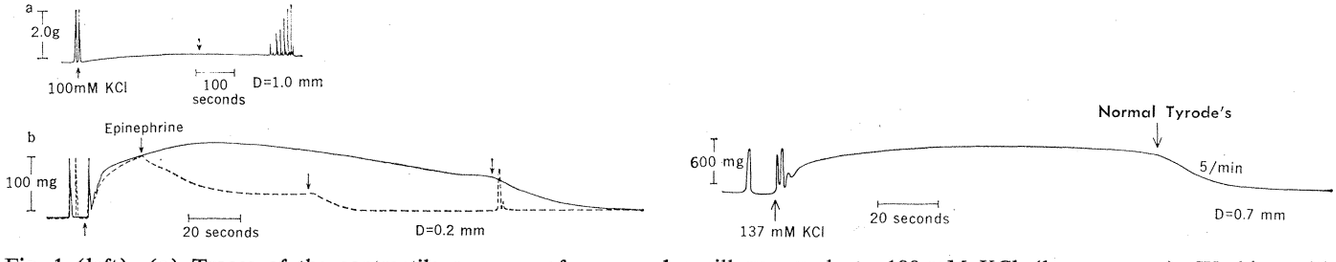

Fig. 1 (left). (a) Traces of the contractile response of a normal papillary muscle to 100 m*M* KCl (lower arrow). Washing with normal Tyrode's reverses the response (upper arrow). (b) The superimposed traces of successive experimental runs of a muscle pretreated with reserpine. At the lower arrow 100 m*M* KCl is administered. Contracture tension rises rapidly (solid line) and is maintained until the KCl removal (upper arrow). The broken trace represents the repeat of the experiment, with epinephrine (1 μg/ml) being added at the upper arrow, and with a resultant drop in tension. *D*, diameter of the preparation. Fig. 2 (right). Tracings of twitches (frequency, 5 per minute) and contracture response of a papillary muscle (*D*), in $10^{16}M$ propranalol. Temperature, 26°C.

twitch suggests renewed synthesis and release of catecholamines. This decline in contracture and the shortening of twitch was not observed when the site of drug action was blocked by propranalol.

Since a primary role has been attributed to the sarcoplasmic reticulum in the relaxation process (*6*), it becomes necessary to ask whether the relaxation effect of catecholamines is mediated through the sarcoplasmic reticulum or through some other mechanism. The ease of eliciting contractures in the frog heart, a tissue lacking in sarcoplasmic reticulum (*7*, *8*) could be suggestive. However, it must be remembered that epinephrine also relaxes the contracture induced by KCl in the frog ventricles (*3*). Other biochemical evidence indicates that both reserpine at $4 \times 10^{-4}M$ and propranalol at $4 \times 10^{-3}M$ inhibit the calcium-pumping activity of the isolated vesicles [skeletal muscle (*9*) and cardiac muscle (*10*, *11*)] which was not reversed by catecholamines (*11*). However, the concentrations of these agents used here were of the order of 1000 times less, and propranalol at $10^{-6}M$ had no negative inotropic effect. Furthermore there is no biochemical evidence yet available to suggest an enhancement of calcium-pumping activity of the isolated vesicles in the presence of catecholamines (*10*). In fact there is some evidence that catecholamines may inhibit calicum-pumping of the sarcoplasmic reticular fraction (*12*). Electronmicroscopic examination of the preparations showed no gross differences in the sarcoplasmic reticulum or mitochondrial appearance of hearts treated with reserpine or propranalol when compared to the nontreated myocardium.

The role of catecholamines in the myocardial contractility, regardless of their mechanisms of action, may be intimately related to the process of relaxation. The shortening of the duration of contraction produced by epinephrine, in spite of an increase in the duration of the action potential (*3*, *13*), may well reflect the beat-to-beat relaxation-stimulating action of catecholamines that is analogous to its effect to relax contracture. In the mammalian heart, where the sequestering of calcium must be efficient, the calcium-pump may be doubly protected by: (i) The increased total membrane surface, for example, well-developed sarcoplasmic reticulum and (ii) an abundance of catecholamines through rich sympathetic innervation. It is this very efficient calcium-pump that is able to handle the extra load of calcium during a challenge with the solutions that produce contractures. The diminished sequestering surface (for example, with frog ventricle) or the removal of the "pump-stimulant" (catecholamines) is sometimes sufficient to slow the transport system just enough to throw the muscle into contracture (a state of calcium overload) upon exposure to KCl solutions.

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14. I thank Drs. A. J. Brady and G. Langer for discussions and advice and R. Orkand for reading the manuscript. Supported by American Heart grant 4-444968-58138.

21 April 1969; revised 22 July 1969

Flower of Tetraplasandra gymnocarpa Hypogyny with Epigynous Ancestry

Abstract. *Comparative herbarium studies, floral anatomy, and distributional data show that* Tetraplasandra gymnocarpa, *an araliad with hypogynous flowers, evolved in Hawaii from ancestors with epigynous flowers. Suggested causes for this reversal of a well-known evolutionary trend are (i) isolation of the ancestors from flower-eating predators and (ii) selection for increased outcrossing.*

A flower is hypogynous if its sepals, petals, and stamens radiate from below the locules, or seed cavities. The ovary, which encloses the locules, is said to be superior when the flower is hypogynous. A flower is epigynous if its sepals, petals, and stamens diverge above the locules and therefore appear affixed to the summit of the (inferior) ovary. The change from hypogyny to epigyny, having occurred independently in many families, is one of the firmly established evolutionary tendencies in flowering plants. We report an example of the reverse change, presumably the first example of secondary hypogyny to be recognized (*1*).

The reversal has taken place in the Araliaceae, a predominantly tropical family whose most familiar member (to residents of northern temperate regions) is English ivy. All but a few of the hundreds of species of Araliaceae have markedly inferior ovaries, and completely superior ovaries occur only in *Tetraplasandra*, a genus of trees of Pacific islands.

The geographic distribution of *Tetraplasandra* and comparative anatomy of the flowers (*2*) provide the evidence for the secondary origin of the superior ovary. Twenty species are found only on the Hawaiian islands (*3*); the others—probably only two in number (*4*)—are confined to islands of the western Pacific: New Guinea, Celebes, Palawan in the Philippines, and San Cristóbal in the Solomons (*5*). Because of the relative geologic youth of the Hawaiian chain, one would expect the western species to be older, and floral structure confirms this expectation. Flowers of *T. paucidens* Miq. from New Guinea have the following structural features (Fig. 1) that most plant systematists would accept as primitive: (i) stamens and carpels are numerous (*6*); (ii) carpel margins are unsealed above the locules, and their apical (stigmatic) portions unite around an open stylar canal; (iii) dorsal bundles of the carpels are separate, at least in part, from bundles supplying the sepals, petals, and stamens; (iv) some of the ventral bundles are in pairs. Hawaiian tetraplasandras are thought to have originated from one ancestral colonizing species (*7*), and our studies indicate that *T. hawaiensis* A. Gray is the modern species closest to the ancestor. A species of broad ecological amplitude (*8*), *T. hawaiensis* is found on four of the Hawaiian islands; moreover, it has many resemblances to tetraplasandras of the western Pacific, including the floral characters listed above (*9*).

Flowers of *T. hawaiensis*, like those of the western Pacific species, are epigynous. There are two species of *Tetraplasandra* endemic to Oahu, however, in which the ovaries are superior at the time of flowering and remain