This experiment depends on the cerebellar peduncles having been cut so each experimental brain was examined very carefully to ensure that the pedunculotomy was total. Any brains in which there was the slightest doubt were discarded. The overwhelming impression from the experimental results is that regardless of the age at operation the overall density of the blood vessels in the cerebellar cortex was the same as in the control animals. However, the arrangement of blood vessels was much more disorganized and the clear palisadal appearance in the molecular layer had been lost. The vessels in the granular layer were also more irregularly arranged. The Purkinje cell layer was always seen to have a good blood supply in all the experimental animals. Large vessels were seen to run either from the pial surface or the white matter core towards the PC layer, and an anastamotic network was clearly visible. Sometimes vessels were seen to link the white matter in the core of the folium to the pial surface, although with this type of experiment it is impossible to say in which direction the blood was flowing. Reinforcement of the blood supply to the cerebellum from unusual sources was also sometimes seen, with vessels linking the tectum or the dorsal surface of the brainstem to the cerebellar cortex. These were never seen in the control animals. Examples of the appearances seen in both control and experimental animals are shown in the figure.

The conclusion that can be drawn from the experiment is that whatever the effect of the operation on the subsequent growth of the cerebellum and its constituents it is unlikely to be because of a reduced vascularity within the cerebellar cortex. In particular the changes observed in the Purkinje cells[5] are probably not caused by a compromised blood supply. This means that the operation of pedunculotomy is a useful, specific method of deafferenting the cerebellar cortex and therefore can be used to study further the effect of removal of afferents in the subsequent development of neuronal tissue. If the operation is carried out bilaterally it gives an in vivo tissue culture which has its own blood supply for the study of the effect of different substances such as drugs or hormones on the development of neonatal neural tissue.

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## Excitation-contraction coupling in the myocardium of hibernating chipmunks

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*Summary.* In the myocardium of nonhibernating chipmunks, replacing external Ca by Sr markedly prolongs the action potential plateau with an increase in contraction, while in preparations from hibernating animals this procedure inhibits both responses. Pretreatment with 4-aminopyridine causes a prolongation of the action potential plateau by Sr in hibernating animals.
*Key words.* Hibernating chipmunks; myocardium; plateau potential; slow inward current; strontium; 4-aminopyridine.

It has recently been demonstrated that some characteristics of cardiac excitation-contraction coupling are markedly changed during hibernation[1]. Namely, the shape of the myocardial action potential of a hibernating animal is different from that of a nonhibernating animal. In hibernating animals the action potential and the slow action potential show a reduced amplitude of the plateau phase[1]. Such characteristics of the action potential of the myocardium of hibernating animals may be explained by either a smaller contribution of the slow inward current or a greater contribution of the transient outward current which masks the slow inward current[2-4]. In the present experiments, therefore, the effects of replacing external Ca by Sr and the application of 4-aminopyridine (4-AP) were examined by using microelectrode techniques, because Sr permeates the slow calcium channels but strongly reduces the rate of inactivation of the slow inward current and the potassium outward current, and 4-AP inhibits the transient outward current.

*Materials and methods.* Asian chipmunks *(Tamias sibiricus)* of either sex were trapped in September and transferred to individual wire mesh cages. Some of them were kept in a room controlled at 25 °C and used for experiments on nonhibernating preparations. Others were introduced to a darkened cold room (4 ± 1 °C) with food (a standard diet of pelleted laboratory rat chow) and water available. Most of them had exhibited preliminary bouts of hibernation within 3 weeks and subsequently they showed several consecutive bouts of hibernation of more than 1 week duration. Animals during deep hibernation were used for experiments on hibernating preparations. Animals were killed by a blow on the head. The heart was quickly excised, and a papillary muscle, 2–3 mm in length and less than 1 mm in diameter, was isolated from the right ventricle. The preparation was mounted with the ends impaled on two hooks, one of which was attached to a force displacement transducer, and equilibrated for 2 h in a tissue bath containing Krebs-Ringer solution aerated with 95% $O_2$ and 5% $CO_2$[1]. The composition of the Krebs-Ringer solution in millimoles per liter was: NaCl, 120; KCl, 4.8; $CaCl_2$, 1.2; $MgSO_4 \cdot 7\ H_2O$, 1.3; $KH_2PO_4$, 1.2; $NaHCO_3$, 24.2; and glucose, 5.5 (pH 7.4). When external $Ca^{2+}$ was replaced by strontium, 1.2 mM $CaCl_2$ was replaced by 2 or 4 mM strontium chloride. The temperature of the superfusate was maintained at 30 °C. The preparations were stimulated at 0.2 Hz with pulses 1 ms in duration and twice the diastolic threshold.

Membrane action potential was recorded through glass microelectrode filled with 3 M KCl. The action potential and the mechanical tension were simultaneously displayed on a storage oscilloscope (Tektronix 7613) and recorded on FM tapes (Sony recorder FE 3500).

*Results and discussion.* In papillary muscles of nonhibernating animals, the maximum level of the plateau phase of the action potential reached +15 ± 3.7 mV (n = 10), similar to that observed in mammalian cardiac muscles of other species. In such preparations, the effects of Sr, a divalent cation which traverses the slow inward current channels[5,6] and activates contraciton, were examined. A representative result is shown in figure 1. When the preparations were superfused by the medium containing 2 mM Sr, the contraction was markedly increased (441 ± 40.6% of control, n = 5) and the action potential plateau (APD 80: the action potential duration from the upstroke to 80% of repolarization) was strikingly prolonged to 456 ± 35.7% of control (n = 5). These effects may be explained by the slowing of the rate of inactivation of the slow calcium inward current ($I_s$)[5,7-9] and the blockade of the potassium outward current[3,10] by Sr, both of which result in an increase in $I_s$. The increased flow of Sr through $I_s$ channels might, in turn, enhance the contractile force. In fact, a good correspondence was observed between the magnitude of increase in the contraction and prolongation of APD 80. The present results indicate that $I_s$ is of prime importance in the genesis of the action potential plateau and the activation of contraction of this preparation. The previous result[1] also suggested that in nonhibernating-animal preparations, calcium influx across cell membranes makes a greater contribution to the activation of contraction. In the preparations from hibernating animals, the initial rapid phase of the action potential quickly repolarized to −35 ± 2.0 mV (n = 10), near or below the known threshold potential for $I_s$ in mammalian cardiac muscles[11,12], and the following plateau phase showed a low amplitude. When such preparations were superfused by the medium containing 4 mM Sr, the action potential plateau was markedly shortened (37.5 ± 4.3% of control of APD 80, n = 5) and also the contraction was greatly reduced (4.9 ± 0.8% of control, n = 5) (fig. 2A). Thus, Sr would seem to be unable to carry the inward current which generates the action potential plateau of this preparation, indicating a reduced contribution of $I_s$ through calcium channels to this plateau potential rather than the masking of $I_s$ by the outward current. This reduced contribution of $I_s$ may be explained by either a smaller number of calcium channels or the inhibition of activation of $I_s$ by the development of transient outward current. To clarify these two possibilities, the effect of Sr was examined in the presence of 4-aminopyridine (4-AP), an inhibitor of the transient outward current[13] (fig. 2B). In the presence of 1 mM 4-AP, the early plateau phae of the action potential was observed. In such preparations, 4 mM Sr-medium with 4-AP markedly prolonged the action potential plateau (355 ± 41.8% of control of APD 80, n = 5) in much the same way as observed in preparations from nonhibernating animals, suggesting the involvement of the transient outward current in the reduced contribution of $I_s$. These results indicated that $I_s$ of the present preparations is rapidly offset by development of the transient outward current, resulting in less activation of $I_s$. Thus, the low amplitude of the plateau potential of hibernating animals is, at least in part, due to the inhibition of $I_s$ activation by a large transient outwárd current. This transient outward current is a consistent feature of the early outward current found in sheep cardiac Purkinje fibers[13], which is blocked by 4-AP at voltages more positive than −40 mV. The inhibition of $I_s$ activation may not be attributed to raised intracellular Ca concentration which causes a faster inactivation of $I_s$ and an increase in potassium outward current leading to a faster repolarization, since Ca replacement by Sr failed to slow the rapid repolarization. In addition, it is interesting to note that in Sr-medium with 4-AP the developed tension of cardiac muscle of hibernating animals was not increased even though the action

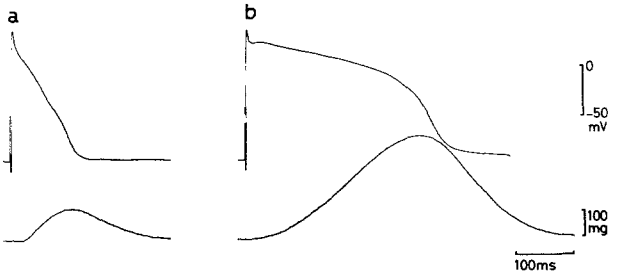


Figure 1. Effects of Ca replacement by Sr on membrane action potential and the contraction of papillary muscles from nonhibernating animals. Ca was removed from the medium and replaced by 2 mM Sr. Upper trace shows the action potential, and lower trace shows the contraction. a: control, b: 10 min after Ca replacement by Sr.

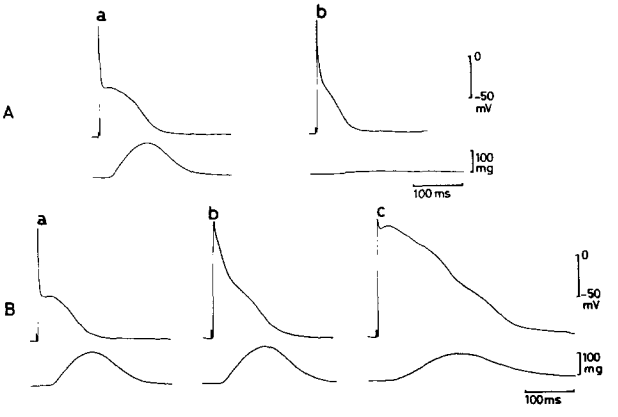


Figure 2. Effects of Ca replacement by Sr in the absence (A) and the presence (B) of 4-aminopyridine (1 mM) on membrane action potential and the contraction of papillary muscles from hibernating animals. Ca was removed from the medium and replaced by 4 mM Sr. Upper trace shows the action potential, and lower trace shows the contraction. In *A*, a: control; b: 15 min after Ca replacement by Sr. In *B*, a: control; b: 5 min after application of 4-aminopyridine; c: 15 min after additional Ca replacement by Sr with added 4-aminopyridine.

potential plateau was markedly prolonged. This uncoupling of membrane excitation and the contraction in cardiac muscles of hibernating animals may be explained by the previous assumption that the intracellularly derived calcium plays a more important role in the excitation of contraction than does transsarcolemmal calcium influx[1]. However, further study is necessary to clarify the precise mechanism of this action.

The present study suggests that a flow of $I_s$ through calcium channels does not play a major role in regulating the plateau potential of hibernating animal myocardium. It is assumed that there are two currents which may account for this plateau potential. One is the transient inward current carried through nonspecific surface membrane ion channels[14-16], and another is the electrogenic Na-Ca exchange inward current resulting from Ca moving out of the cell in exchange for external Na[17,18]. It is well known that both the transient inward current and the Na-Ca exchange current would be linked to an increase in intracellular calcium activity through a process of release from internal stores[14-18]. In addition, the developed tension in hibernating animal myocardium depends on Ca released from internal stores[1]. Thus, the change in the plateau potential of myocardium from hibernating animals may be due to a larger contribution of Ca released from internal stores which causes a greater transient inward current. This is also in good agreement with the previous result that ryanodine, an inhibitor of internal Ca release, markedly inhibited the action potential plateau of this preparation[19]. The finding that the contribution of $I_s$ to the cardiac action potential was reduced during hibernation may have important

implications for understanding the unique characteristics of myocardium from hibernating animals, such as the cold tolerance and the markedly different effects of cardioactive agents[19–21]. The present result is important for further understanding of the cardiac excitation-contraction coupling.


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## The function of intimal longitudinal smooth muscles of the human coronary artery[1]

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*Summary.* Bundles of smooth muscles in the intimal layer of the human coronary artery contracted in a longitudinal direction on the application of vasoactive substances. The data indicate that the human coronary artery contracts not only transversely but also longitudinally.
*Key words.* Human coronary artery; smooth muscles in intimal layer; longitudinal contraction.

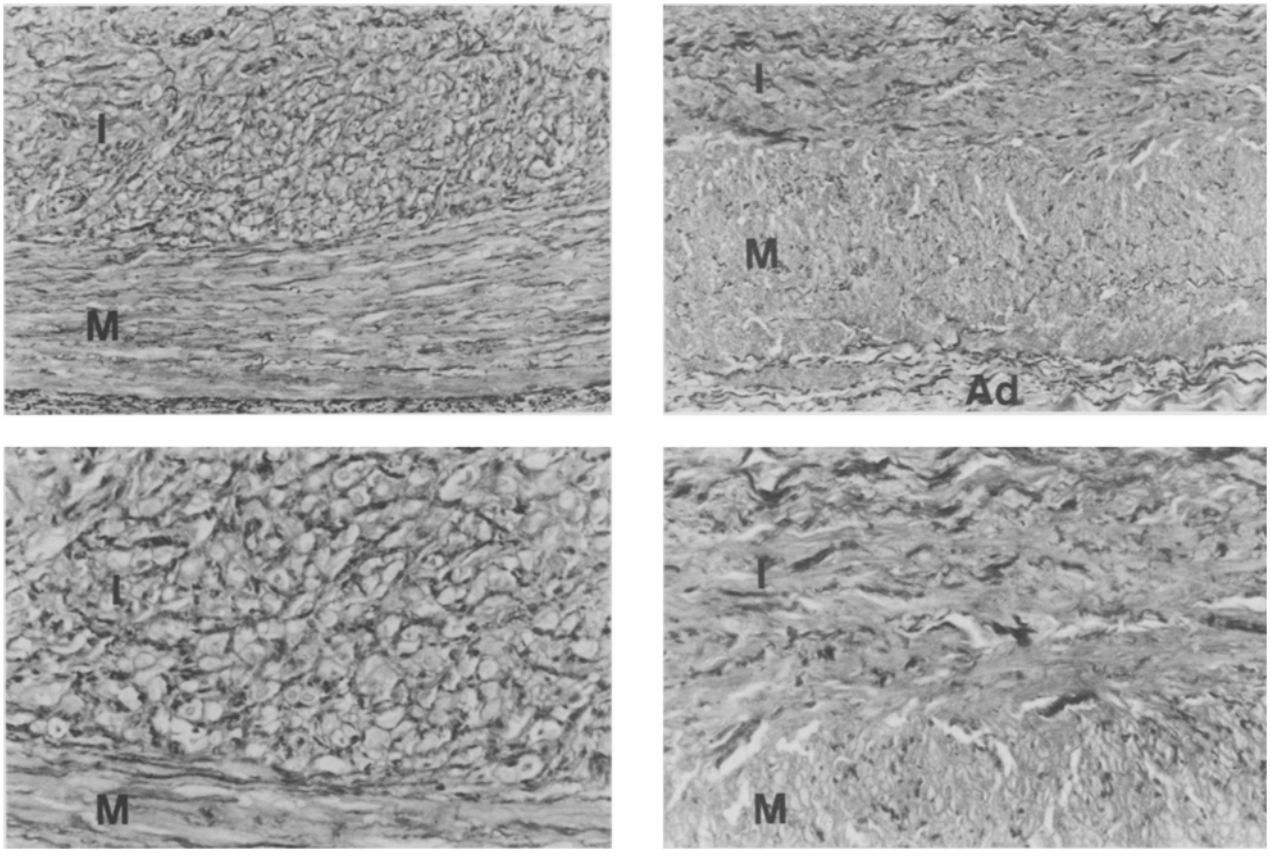


Figure 1. Left: a cross-section of human coronary artery for light microscopy. low magnification, × 35 (top); high magnification, × 70 (bottom). Right: a longitudinal-section of the same specimen. low magnification × 35 (top); high magnification, × 70 (bottom). Smooth muscle cells of the intima are arranged nearly parallel to blood stream. I, intima; M, media; Ad, adventitia. (elastica Van Gieson stain).