PROLONGATION OF THE ACTION POTENTIAL PLATEAU OF EMBRYONIC CHICK HEARTS ORGAN CULTURED IN THE PRESENCE OF CYCLIC AMP

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We have previously demonstrated that young (2-3 day-old) embryonic hearts, prior to innervation, possess only tetrodotoxin (TTX)-insensitive slow Na⁺ channels (1, 2). TTX-sensitive fast Na⁺ channels are acquired during development, and old (14-18 days) embryonic hearts possess a high density of such channels. During organ culture for 1-2 weeks, young embryonic hearts retain their slow Na⁺ channels and do not gain fast Na⁺ channels as they do during in situ development (3, 4). Minced fragments of old hearts, which normally do not have extensive slow Na⁺ channels, acquire slow Na⁺ channels during organ culture (3).

A relationship appears to exist between the cyclic AMP level and slow channel availability. The basal cyclic AMP level in young embryonic chick hearts is much higher than in old embryonic hearts (5). Further, we demonstrated that cyclic AMP or dibutyryl cyclic AMP mimicked the effect of catecholamines and methylxanthines in making an increased number of slow Na⁺-Ca⁺⁺ channels available in myocardial cells (whose fast Na⁺ channels were inactivated with TTX or in 27 mM K⁺), except that the action of cyclic AMP or dibutyryl cyclic AMP was much slower in onset (6, 7). Oscillations in cyclic AMP levels also occur during each cardiac cycle (8, 9). Denervation of skeletal muscle produces an increase in cyclic AMP (10), and the action potential becomes resistant to TTX, although this change is prevented by protein synthesis inhibitors (11).

Since these observations suggest that there might be some correlation between the number of slow cation channels available and cyclic AMP level and slow channel availability. The basal cyclic AMP level in young embryonic chick hearts is much higher than in old embryonic hearts (5). Further, we demonstrated that cyclic AMP or dibutyryl cyclic AMP mimicked the effect of catecholamines and methylxanthines in making an increased number of slow Na⁺-Ca⁺⁺ channels available in myocardial cells (whose fast Na⁺ channels were inactivated with TTX or in 27 mM K⁺), except that the action of cyclic AMP or dibutyryl cyclic AMP was much slower in onset (6, 7). Oscillations in cyclic AMP levels also occur during each cardiac cycle (8, 9). Denervation of skeletal muscle produces an increase in cyclic AMP (10), and the action potential becomes resistant to TTX, although this change is prevented by protein synthesis inhibitors (11).

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The embryonic hearts were removed at 2-3 days (entire hearts were used) or at 14-15 days (ventricles were minced into small pieces, approximately 1 mm³), and organ cultured for 1 to 7 days in normal culture medium or medium containing cyclic AMP (1 mM; 0.5 mM theophylline was added to retard the degradation of cyclic AMP by phosphodiesterase) or...
Table 1. Electrical parameters of the action potential recorded from hearts organ cultured in cyclic AMP-containing medium or in high K⁺ medium

| Preparation              | Treatment                  | Period of Culture (Days) | N  | Resting Potential (mV) | dVmax (V/sec) | Action Potential Duration 50% (msec) | 90% (msec) |
|--------------------------|----------------------------|--------------------------|----|------------------------|---------------|-------------------------------------|-----------|
| 2–3 Day-Old Whole Hearts | None                       | 6–8                      | 14 | 62 ± 2                 | 12 ± 2        | 111 ± 3                             | 139 ± 4   |
|                          | Cyclic AMP+ Theophylline   | 6–7                      | 7  | 67 ± 4                 | 19 ± 2        | 180 ± 16                            | 204 ± 15  |
|                          | High K⁺                    | 6–7                      | 6  | 59 ± 2                 | 12 ± 1        | 94 ± 10                             | 138 ± 13  |
| 14–15 Day-Old Minced Fragments | None                     | 1–3                      | 51 | 69 ± 1                 | 53 ± 5*       | 122 ± 2                             | 154 ± 3   |
|                          | Cyclic AMP+ Theophylline   | 1–2                      | 10 | 70 ± 2                 | 72 ± 12*      | 217 ± 8                             | 263 ± 10  |
|                          | High K⁺                    | 2–4                      | 7  | 74 ± 1                 | 124 ± 13      | 135 ± 15                            | 162 ± 16  |

The cyclic AMP-containing culture medium contained 1 mM cyclic AMP plus 0.5 mM theophylline. The high K⁺ culture medium contained 50 mM K⁺.

The membrane potentials were recorded in normal medium not containing cyclic AMP or extra external K⁺. Values given are the means ± 1 SE. N gives the number of preparations tested.

* These dVmax values fell into a wide range.

Fig. 1. Illustration of the effect of chronic exposure to cyclic AMP (1 mM) plus theophylline (0.5 mM) on organ-cultured hearts. A-B: Minced fragments from 15 day-old hearts cultured for 24 hr. A: Control action potential recorded from tissue not exposed to cyclic AMP. B: Prolonged action potential recorded from tissue exposed to cyclic AMP. C-D: 3 day-old whole hearts cultured for 7 days. C: Control action potential recorded from a heart not exposed to cyclic AMP. D: Prolonged action potential recorded from a heart exposed to cyclic AMP. The records in B and D were taken with the hearts bathed in normal medium not containing cyclic AMP and theophylline. dV/dt is given by the upper traces; the dV/dt trace was purposely not aligned with the V-t trace so as not to obscure dV/dt within the rising phase of the action potential. Voltage, dV/dt, and time calibrations in D apply to all panels.

High K⁺ (50 mM); culture methods were previously described in detail (3). Cyclic AMP and theophylline are stable during organ culture. After the period in organ culture, the hearts were removed and placed in a tissue bath containing normal Puck’s medium at 37°C, and conventional intracellular microelectrode recordings were performed.

The most prominent and pronounced change in the action potential in hearts exposed
to cyclic AMP during organ culture was the prolongation of the plateau component (phase 2). This was true both in the case of the young embryonic hearts and the old minced fragments. There was an increase in both the 50% duration and the 90% duration of the action potential (Table 1). The 50% duration increased from 122 msec to 217 msec for the minces, and from 111 msec to 180 msec for the young hearts. Typical action potentials recorded from hearts with and without exposure to cyclic AMP during organ culture are illustrated in Fig. 1. As shown, the prolongation of the action potential is due to increase in duration of the plateau phase. The duration of the action potentials obtained from hearts organ cultured in high K+ media were not significantly different from those of hearts cultured in normal medium (Table 1). In chick hearts left in situ, the duration of the action potential remains essentially unchanged during development; the overall mean for all ages was 105 msec at 50% repolarization and 130 msec at 90% repolarization (2).

The resting potentials of the cyclic AMP-treated groups and high K+-treated groups were not significantly different from the control groups, both in young hearts and old minced fragments (Table 1). There was great variability in the maximum rate of rise (+V\text{max}) for the action potential obtained from the old minced fragments, as previously reported (3). The minced fragments exposed to cyclic AMP had a higher average +V\text{max} than the control group, but the fragments exposed to high K+ had an even higher value (Table 1). In young hearts, +V\text{max} remained at the low value of 12 V/sec after organ culture in normal medium thus confirming our previous conclusion that fast Na+ channels do not appear while in vitro organ culture (3). Exposure to high K+ had no effect on +V\text{max}, but there was a significant increase in +V\text{max} in the cyclic AMP-treated group (Table 1). Short-term exposure to cyclic AMP plus theophylline did not prolong the action potentials.

It is likely that the long-term application of cyclic AMP acted on the cell membrane directly or indirectly to increase the inward cation charge transfer responsible for the plateau component. It is possible that cyclic AMP increases the density of available slow cation channels during organ culture, and thus prolongs the action potential plateau. The small increase in +V\text{max} also observed is consistent with an increase in density of available slow cation channels (presumably Na+ or Na+-Ca++ channels).

The present results are consistent with our previous finding (6) that cyclic AMP or dibutyryl cyclic AMP slowly made an increased number of slow cation channels available in old intact embryonic hearts (whose fast Na+ channels were inactivated with TTX) which were not blocked by the beta-adrenergic blocking agent, propranolol. Tsien (14) demonstrated by iontophoretic injection of cyclic AMP intracellularly that the ionic currents associated with the cardiac action potential plateau are sensitive to changes in cyclic AMP levels. Of course, other possibilities also exist for the prolongation of the action potential by chronic exposure to cyclic AMP, including an effect on the K+ conductance changes. The increase in +V\text{max} observed in the cultured minced fragments of old hearts exposed to high K+ suggests that the injury incurred during mincing and consequent diminution in +V\text{max} is minimized by either of these treatments. Fresh non-cultured intact 14-15 day-old hearts have an average +V\text{max} of about 130 V/sec.
The results also confirm and extend our previous finding (3) that fast Na\(^+\) channels do not appear in young hearts during the \textit{in vitro} organ culture as normally occurs during \textit{in situ} development. The present results demonstrate additionally that neither addition of exogenous cyclic AMP plus theophylline (to elevate internal cyclic AMP levels) nor elevation of \([K^+]_o\) (to stimulate non-specific protein synthesis) act to promote acquisition of fast Na\(^+\) channels in young hearts. These findings suggest that something peculiar to the \textit{in situ} condition of the heart exerts major control over the development of fast Na\(^+\) channels. The possibility that the arrival of the innervation exercises one such control remains as a likely one. Even organ culturing young hearts on the chorio-allantoic membrane (CAM) of other chick embryos, to make the culture condition closer to that \textit{in situ}, does not allow the fast Na\(^+\) channels to develop (4). It is interesting, however, that the mean action potential duration of hearts cultured on the CAM was double that of hearts cultured in glassware \textit{in vitro} in normal medium (240 msec vs 120 msec). That is, culturing young hearts on the CAM gave similar results as culturing hearts in glassware in the presence of cyclic AMP plus theophylline, with respect to prolongation of the action potential plateau. It is possible that the blood perfusion of the CAM provides the cultured heart with a substance which increases the intracellular cyclic AMP level.

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