Developmental Changes of the Alpha1-Adrenergic System in the Rat Heart

한 형 미
국립보건안전연구원

The effect of alpha$_1$-adrenergic stimulation on cardiac automaticity changes during myocardial development. To illustrate, in isolated tissues from the mature canine heart, alpha$_1$-adrenergic stimulation usually induces a decrease in automatic rate. On the other hand, many early neonatal fibers exhibit an increase in automatic rate in response to alpha$_1$-agonists, which is not seen in the adult.

We hypothesized that sympathetic innervation might have a role in changing the responsiveness to alpha$_1$-adrenergic stimulation from positive to negative chronotropy. This was based in part on the similar time course for developmental changes in cardiac innervation and in automatic responsiveness. It was also based in part on the understanding that the early neonatal heart, which tends to show a positive chronotropic response to alpha$_1$-stimulation, is poorly innervated by cardiac sympathetic nerves, and that the rapid postnatal development of innervation might modulate the change in the chronotropic response. In the present study, we tested our hypothesis by observing the effects of alpha$_1$-adrenergic agonists on automaticity of neonatal rat myocytes in culture alone and in co-culture with sympathetic ganglion cells (nerve-muscle co-cultures, NM co-cultures). To test whether this model was analogous to the *in situ* heart, we also performed studies on newborn and adult rat ventricle. In neonatal rat ventricle, *in vitro* phenylephrine ($1X10^{-8}$ M) induces an increase in automatic rate from 115±12 (mean±SEM) to 168±10 beats/min, $P<0.05$. In contrast, in adult rat ventricle, the rate decreases

from 36±8 to 12±12 beats/min, P<0.05. At both ages, the response is attenuated by alpha$_1$-antagonist, prazosin ($1X10^{-6}$ M). We next used cultures of neonatal rat myocytes to determine whether maturation of innervation contributes to the ontogeny of this response. All non-innervated cultures showed a positive chronotropic response to alpha$_1$-stimulation; phenylephrine ($1X10^{-6}$ M) increased the rate from 40±2 to 52±2 beats/min, P<0.05. In contrast, 60% of the myocytes innervated with sympathetic neurons showed a decrease in rate in response to phenylephrine, from 78±6 to 67±6 beats/min, P<0.05. These results demonstrated that the modulation of the myocardial response to alpha$_1$-adrenergic stimulation by the occurrence of innervation in tissue culture. This provides an explanation for the previously identified ontogenic change in alpha$_1$-adrenergic effects on intact cardiac fibers from excitation to inhibition.

We next tested the hypothesis that this neurotrophic effect might be a result of a humoral substance into the bulk phase of the culture medium by neurons. The negative chronotropic response to alpha$_1$-stimulation persisted in NM co-cultures pretreated with the mucarinic antagonist, indicating that acetylcholine is not the trophic agent. Further, variations in the concentration of norepinephrine, epinephrine or dopamine in the NM culture media do not account for the presence of a negative chronotropic response to alpha$_1$-stimulation. Finally, muscle cells grown in the same Petri dish with innervated muscle cells, to allow conditioning of the muscle cell environment by the neuron, do not acquire a negative chronotropic response to alpha$_1$-stimulation. These results demonstrated that this trophic effect was not due to the release of a humoral substance into the bulk phase of the culture medium, but requires close nerve muscle association.

As a next step, we examined whether there is any developmental change in the alpha$_1$-adrenergic receptor complex in terms of coupling to the components involved in signal transduction. Among several possible components, we tested whether a pertussis toxin (PT)-sensitive guanine nucleotide regulatory protein (G protein) is

coupled to alpha$_1$-adrenergic receptor complex in the neonatal and adult rat heart. To investigate the possibility of a developmental change in coupling of a PT-sensitive G protein to the alpha$_1$-adrenergic receptor, radioligand binding experiments with the iodinated alpha$_1$-selective radioligand [^{125}I]-I-2-[beta-(4-hydroxyphenyl)ethylamino-methyl]tetralone ([^{125}I]-IBE 2254) were performed on membranes prepared from control and PT-treated neonatal and adult rat hearts. Scatchard analysis showed fewer alpha$_1$-adrenergic receptors in the adult than in the neonate (168±10 fmol/mg protein in the neonate vs. 124±13 fmol/mg protein in the adult), but similar affinities (equilibrium dissociation constant: 124±29 pM in the neonate vs. 140±34 pM in the adult). PT treatment did not alter the results. In both the neonate and adult, 5'-guanylylimidodiphosphate [Gpp(NH)p, 500 uM] shifted the *l*-epinephrine competition curve to the right and increased the slope factor toward unity. PT had no effect on the *l*-epinephrine competition curve in the neonate. However, in the adult, PT itself caused a partial shift in the agonist competition curve, reducing but not eliminating the effect of Gpp(NH)p. Consistent with the results from the binding experiments, PT did not have any effect on the alpha$_1$-adrenergic-mediated positive chronotropic response in the neonate, whereas in the adult the alpha$_1$-adrenergic-mediated negative chronotropic response was completely converted to a positive one after PT treatment. These results indicate the presence of a PT-sensitive G protein linked to the negative chronotropic response during development.

In summary, these studies demonstrated that 1. there was a developmental change in alpha$_1$-mediated cardiac chronotropic response from positive to negative in the rat heart, 2. sympathetic innervation might be responsible for this developmental change in alpha$_1$-mediated cardiac automaticity and 3. a PT-sensitive G protein became linked to alpha$_1$-adrenergic receptor during development in the rat heart.