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Review

Autonomic receptor–effector coupling during post-natal development

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1. Introduction

There are numerous age-dependent changes in the autonomic responsiveness of ventricular myocardial or conducting tissue, including effects on chronotropy, inotropy and repolarization. While some of these involve a simple quantitative difference in the magnitude or sensitivity of the response, in other cases a more complex qualitative alteration is observed [1–6]. In fact, these qualitative differences in the nature of the response with age often involve diametrically opposed responses in the newborn and adult. It has become clear in recent years that many of the opposing and seemingly conflicting actions of autonomic agonists during development can only be understood after careful consideration of the issues of drug–receptor interaction and receptor–effector coupling [2,7–11]. Thus, a full understanding of the age-dependent actions of any particular autonomic agonist requires consideration of the various receptor subtypes with which that agonist may interact, and the associated signal transduction cascades that may be functional at different stages of development.

The current review will therefore primarily focus on those cardiac responses to autonomic agonists which exhibit a qualitative, and not just quantitative, change with age. Data will be reviewed for the α_1- and β-adrenergic, and the muscarinic cholinergic systems. In addition, where data are available, the nature of the developmental regulator that controls the change in autonomic responsiveness will be discussed. In particular, the question will be asked whether the onset of innervation, and in particular sympathetic innervation, may serve as a developmental stimulus in each case.

2. Time course of innervation

Since one of the issues being addressed in this review is the role played by innervation as a developmental regulator of cardiac autonomic responsiveness, it is helpful to review the time course of cardiac parasympathetic and sympathetic innervation. For the sake of simplicity, only the rat heart will be considered, since much of the data on autonomic responsiveness during development cited herein is derived from that species. Parasympathetic innervation of the rat heart (as measured by choline acetyltransferase activity) is first detected a few days before birth [12]. Maturation of atrial innervation appears to be complete 30–60 days post-natal (for review, see [13]). Sympathetic innervation begins later, and Fig. 1 summarizes data from various sources on the time course in the rat ventricle. For the first week after birth the rat ventricle lacks any evidence of sympathetic innervation, regardless of the parameter used to assess innervation. The earliest indication of the onset of innervation is provided by studies with radioactively labeled norepinephrine (upper of the three horizontal bars in Fig. 1). Uptake of exogenous norepinephrine (measured 30 min after injection) is detectable in the second week, but retention (defined as the amount remaining 3 h after injection, relative to the 30-min timepoint) is not evident until the third week. Adult characteristics are achieved after 5 weeks [14]. Nerve function (lower bar in Fig. 1) appears after norepinephrine uptake. Using either tyramine or field stimulation to release catecholamines and elicit an inotropic response, an effect first appears at the beginning of the third week (but see [15] for evidence that releasable catecholamine may be present in the ventricular septum immediately after birth). The response matures

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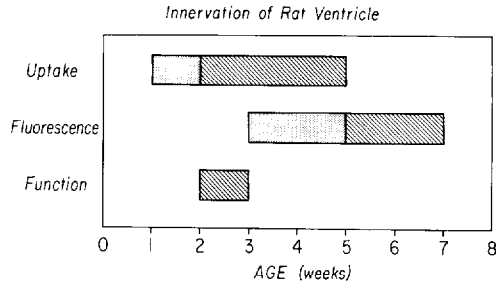

Fig. 1. Time course of sympathetic innervation in the developing rat ventricle. The data represented were integrated from several different sources (see text). In each case, the horizontal bar marks the time period between when a particular parameter first is detected and when that parameter achieves adult or near-adult values. Where the bar is subdivided into two regions, the division marks the time when the parameter qualitatively matches adult characteristics, while the end of the line indicates quantitative agreement with adult characteristics as well.

rapidly, reaching to near adult levels by the end of that week [5]. Catecholamine fluorescence (middle bar of Fig. 1) is the least sensitive assay of the early stages of innervation, not being observable until 3 weeks after birth. An adult pattern of distribution is seen after 5 weeks. Intensity continues to increase until the seventh week [16], after the tracer and functional studies have reached a maximum, and suggests that this criterion may be more useful as a guide to later stages of innervation. Thus, although the precise time of sympathetic innervation depends on the parameter studied, by any criterion the newborn rat ventricle provides a convenient extra-uterine source of cardiac tissue which has not yet been sympathetically innervated, and any developmental changes in autonomic responsiveness occurring during the first month of life would be temporally associated with the onset of innervation. Further, by preparing primary cell cultures from neonatal rat ventricles and maintaining them in the presence or absence of neuronal cells, several laboratories have been able to assess in a direct manner the importance of sympathetic innervation to the development of specific myocardial cell physiological and pharmacological characteristics [17–20].

Finally, it is interesting that studies on the developmental expression of α_1-adrenergic, β-adrenergic and muscarinic receptors all suggest a peaking of receptor levels around the first or second week after birth (for review, see [13]), at a time when innervation is maturing rapidly. However, age-dependent differences in relative expression of α_1- or β-adrenergic subtypes have not been observed to date [7,11]. In addition, while limited data are available on age-dependent expression of specific G proteins in the heart [21–24], other evidence suggests that receptor–G protein coupling may be developmentally regulated independent of the absolute levels of the individual proteins [25–27].

3. Alpha-adrenergic responsiveness

α-Adrenergic agonists have been reported to modulate cardiac inotropy, chronotropy and multiple ionic currents (for review, see [28]). We are concerned here only with those effects which differ in the young and mature animal. In this respect, the α_1-adrenergic effect on spontaneous rate is the best characterized. When the ventricular septum is isolated from the newborn rat heart and placed in a tissue bath it beats spontaneously. α_1-Adrenergic agonists cause an increase in rate, or a positive chronotropic response. In contrast, when a similar experiment is carried out on the septum of the adult rat ventricle, the result is a decrease in spontaneous rate, or a negative chronotropic response [18]. A qualitatively similar phenomenon is observed when the experiment is performed using canine Purkinje fibers, rather than the rat ventricular septum [29]. What is particularly interesting is that this developmental shift in α_1-adrenergic responsiveness, from positive to negative chronotropy, can be reproduced in cell culture by the simple expedient of adding sympathetic neurons. Monolayer cultures of newborn rat ventricle cells are spontaneously active. Addition of the α-adrenergic agonist phenylephrine at concentrations of 10^{-9} to 10^{-7} M causes an increase in spontaneous rate. However, if the ventricle cells are maintained in culture with dissociated neurons from the rat sympathetic chain, and these neurons are allowed to functionally innervate the myocardial cells, subsequent exposure to phenylephrine over the same concentration range now causes a decrease in spontaneous rate. This altered responsiveness is not due to a presynaptic action of phenylephrine at the neuron, but rather an altered responsiveness at the myocardial cell as a result of the trophic influence of the neuronal cells [18].

Further, the positive and negative chronotropic responses involve distinct signal transduction cascades. Experiments with the cell culture model demonstrated that the post-innervation negative chronotropic α_1-adrenergic response involves a guanine nucleotide regulatory protein (G protein) which is a substrate for pertussis toxin (PT), but the positive chronotropic response does not require such a G protein [30]. In addition, the α_1-adrenergic receptors in the newborn rat ventricle have been shown in binding assays to couple to a PT-insensitive G protein, with coupling to a PT-sensitive G protein only being detected in the adult [31]. Subsequent intact tissue studies confirmed and extended these observations. It was found that treatment of newborn rats with nerve growth factor (NGF) increases the level of PT ADP-ribosylatable substrate and accelerates the developmental time course of the negative chronotropic α_1-adrenergic response, while treatment with NGF-antibody results in lower levels of the PT substrate and a less mature α_1-adrenergic response [32]. The effect of NGF and NGF-antibody on chronotropic responsiveness is illustrated in Fig. 2. The upper portion of the figure depicts the fraction of preparations that exhibit a

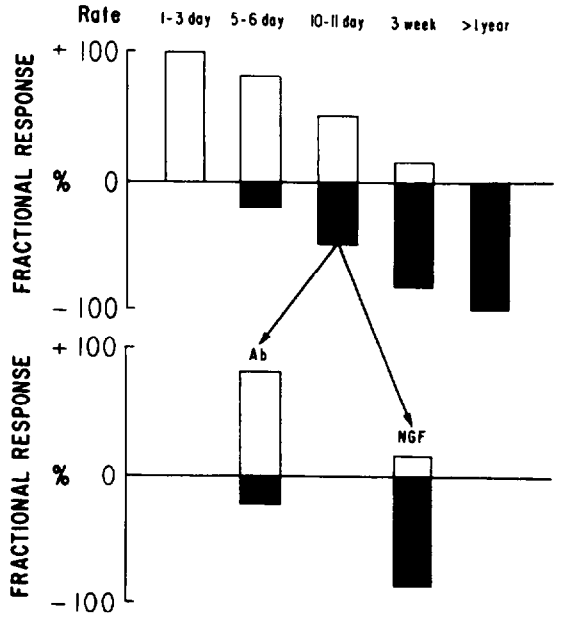

Fig. 2. Fraction of neonatal rat septal preparations which exhibited a negative (filled bar) or positive (open bar) chronotropic response to phenylephrine (10^{-8}–10^{-7}) as a function of postpartum age. In the lower panel, results are shown for rats treated from birth with daily subcutaneous injections of NGF (1.0–1.5 μg) or NGF-antibody (Ab, 10–15 μl; 3–3.5 μg IgG per μl, 20% NGF specific). Fewer animals in the Ab group exhibited a negative chronotropic response, and the fraction that did so was comparable to day 5–6 of the control animals. In contrast, the response of the NGF group was similar to that of the 3-week control group. (Reprinted with permission from Malfatto G, et al. Circulation Research 1990;66:427–437.)

negative (solid bar) or positive (open bar) chronotropic response as a function of age. The lower portion illustrates the characteristics of 10-day-old rats that had been treated either with NGF or NGF-antibody (Ab) from birth. It can be seen from this illustration that the fraction of 10-day-old NGF-treated animals that exhibited a negative chronotropic response was greater than in the control group of a corresponding age, and in fact most closely matched the characteristics of the 3-week-old control rats. In contrast, a relatively small fraction of hearts from the NGF-antibody group of 10-day-old rats showed a negative chronotropic response, and this was comparable to what was observed in 5–6-day-old control hearts.

In addition, the α_1-adrenergic receptors associated with the positive and negative chronotropic responses represent distinct receptor subtypes. While both responses involve α_1-adrenergic receptors, the positive chronotropic response is dependent on a subtype which is sensitive to the agent WB4101 (the so-called α_{1A} subtype), while the negative chronotropic response is dependent on a subtype sensitive to chloroethylclonidine [7] (α_{1B} subtype). Thus it appears in this case that sympathetic innervation results in the expression of a signal transduction cascade which is distinct, both in terms of its receptor subtype and associated G protein, from that which is evident prior to innervation (although the immature cascade is not lost, and in fact is unmasked in the presence of PT, which blocks the mature response [30]). However, several additional factors are worth noting. First, the percentage of α_1-adrenergic receptor binding sites which are sensitive to chloroethylclonidine does not increase with age [7], suggesting that lack of the appropriate receptor protein per se does not explain the absence of the negative chronotropic response in the neonate. Second, while the level of PT-sensitive G proteins has been reported to increase with innervation [30], and the α_1-adrenergic receptors do exhibit coupling to this class of G proteins only in the adult heart [31], immunoblot studies find reduced levels of G_o and G_i with age [21,23]. However, absolute level of G proteins also may not be the limiting factor. When innervated cultures are treated with a low dose of PT to reduce the level of available PT-sensitive G protein to that typical of non-innervated cultures, a negative chronotropic α_1-adrenergic response persists in a fraction of the cultures [27]. This is compatible with the concept that innervation somehow alters the efficiency of receptor–effector coupling. A similar age-dependent increase in the efficacy of receptor–G protein coupling has been reported for the cardiac muscarinic receptor [25,26].

The trophic factor by which innervation exerts its effects on α_1-adrenergic responsiveness is not catecholamines [33], but is in fact neurally released neuropeptide Y (NPY), with the effect requiring sustained pre-exposure to NPY, as would be expected if it were acting as a trophic factor or developmental stimulus [34]. This is shown in the upper panel of Fig. 3, which illustrates the effect of phenylephrine on spontaneous rate in control muscle cultures (MC), and in muscle cultures grown in the sustained presence of NPY. The progressive onset of the effect during 96 h of exposure to NPY is evident from the data in the lower panel of Fig. 3. Furthermore, when innervated cultures were maintained in the continuous presence of the putative NPY antagonist PYX-2 [35] from the time of initial culture, the negative chronotropic α_1-adrenergic response never developed, confirming that neurally released NPY was indeed the stimulus for expression of the mature α_1-adrenergic response.

Spontaneous rate is not the only parameter which is differentially affected by α-adrenergic agonists in the young and mature heart, although it is the only one to be definitively associated with the ontogeny of innervation at this time. It appears that the ability of α-adrenergic agonists to modify the L-type calcium current ($i_{Ca,L}$) also is developmentally regulated. Fig. 4 demonstrates the positive response of the α-agonist phenylephrine on $i_{Ca,L}$ in a neonatal rat ventricle cell in culture. The data were obtained from a neonatal rat ventricle cell grown in monolayer culture, then resuspended on the day of the experiment and studied using the nystatin perforated patch method. The top panel of Fig. 4 depicts the current generated in response to repeated voltage steps from −40 to 0 mV. During the period of superfusion with the α-agonist

phenylephrine (10^{-7} M) there is a marked increase in the current magnitude that rapidly reverses on washout. Full I–V relations during control and phenylephrine (time points *a* and *b*, respectively) are shown in the lower panel. This panel also includes representative current traces during the two conditions.

In a direct comparison of the effect of phenylephrine on $i_{Ca,L}$ in ventricle cells isolated from the newborn and adult rat, Liu et al. found that the current was enhanced only in the newborn [36,37]. In this respect, it is interesting to note that α-adrenergic agonists also stimulate phospholipase C, resulting in formation of IP_3 and diacylglycerol [38], the latter being an activator of protein kinase C. Direct activation of protein kinase C by phorbol esters in neonatal rat ventricle cells results in enhancement of $i_{Ca,L}$ [37,39], similar to the effect of phenylephrine. Also similar to phenylephrine, phorbol esters have no effect on $i_{Ca,L}$ in adult rat ventricle cells [40]. It is possible that the developmental loss of the ability of both α-agonists and phorbol esters to stimulate $i_{Ca,L}$ may be related to developmental changes in protein kinase C isoform expression in the heart [41,42].

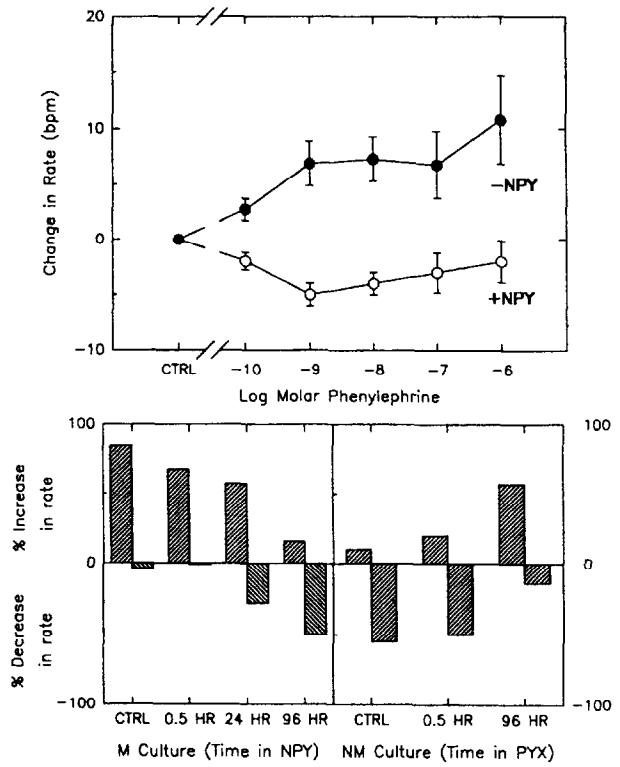

Fig. 3. Effect of NPY and the NPY antagonist PYX-2 on the α_1-adrenergic chronotropic response in neonatal rat ventricle cultures. The upper panel illustrates the effect of growing cultures in the sustained presence of NPY (open circles) on the phenylephrine dose–response relation. Control muscle cultures (filled circles) show a positive chronotropic response, while NPY-treated cultures show a negative chronotropic response. The lower panel groups the cultures on the basis of whether the phenylephrine response (over the concentration range 10^{-9}–10^{-6}M) was positive or negative, and investigates the time course of NPY or PYX-2 treatment. Muscle cultures (left panel) treated acutely with NPY fail to show any negative response (some cultures were non-responding); 24-h NPY treatment leads to a small percentage showing a negative response, and 96-h treatment results in an even greater percentage possessing the negative response. The lower right panel presents data from innervated cultures, which normally exhibit the negative chronotropic response. Acute exposure to the NPY antagonist PYX-2 does not alter the phenylephrine response, but 96-h exposure results in the loss of the negative chronotropic response from most of the innervated cultures. (Reprinted with permission from Sun LS, et al. American Journal of Physiology 1991;261:H969–973.)

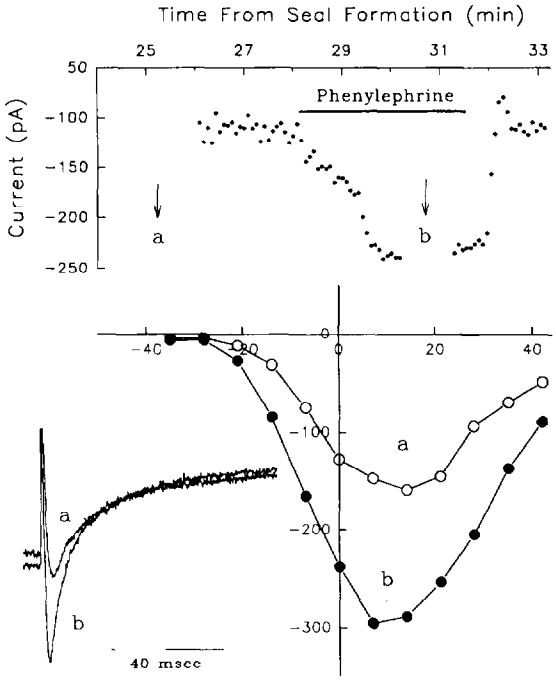

Fig. 4. Effect of phenylephrine on L-type calcium current in a neonatal rat ventricle cell. A monolayer culture was resuspended using a 1–3-min exposure to trypsin, then replated 2–6 h prior to the experiment. A single cell was recorded from using a nystatin-filled perforated patch electrode. The cell was superfused with buffered physiologic salt solution at 32°C, containing 2×10^{-6} M of the β-blocker propranolol. The upper panel shows the time course of the current amplitude from a repetitive series of voltage steps from −40 to 0 mV. During the indicated period, 10^{-7} M phenylephrine was superfused, resulting in an approximate doubling of the current amplitude. The effect of phenylephrine was fully reversible. The lower panel shows the full I–V relations at the times labeled *a* (control) and *b* (phenylephrine) in the upper panel. The inset illustrates the actual current traces during one voltage step in each condition.

While developmental loss of an effect of α-agonists on $i_{Ca,L}$ could contribute to the dominance of the negative chronotropic response with age, it cannot in itself explain a negative chronotropic response. For that effect there must be an action to either decrease an inward current or increase an outward current. Effects on major repolarizing currents typically occur at higher concentrations than those which affect automaticity [40,43], but low concentrations of α-agonists have been reported to stimulate the Na/K pump in adult Purkinje fibers [44], and this could contribute to the negative chronotropic effect. It is not known at this time whether this action of α-agonists is absent in the neonate.

4. Beta adrenergic responsiveness

There are significant age-dependent differences in the electrophysiologic response of the heart to β-adrenergic agonists [45,46], and in the extent to which various ionic currents, including $i_{Ca,L}$ [6] and i_K [47], are modulated. However, in keeping with the overall theme of this review, this section will focus specifically on the relative contribution of β_1 and β_2 receptor subtypes to β-adrenergic responsiveness in the young and adult heart.

The rat heart possesses predominantly β_1-adrenergic receptors, with the β_2 representing from 15 to 25% of the total receptor population both in the young and adult heart [11,48]. Other mammalian species also express both receptor subtypes (see [49] for review). While there have been reports of β_2-mediated electrophysiologic effects in the adult heart [50,51], one study that directly compared neonate and adult found no β_2 effect on action potential configuration at either age in canine Purkinje fibers [52]. In contrast, the β_2-adrenergic receptors appear to couple to inotropic responsiveness, at least in the rat, and there are significant developmental differences in the nature of the response.

In neonatal rat ventricle cells, the response to either β_1- or β_2-adrenergic stimulation is comparable. In both cases, there is an increase in cAMP level, an increase in cell shortening, a more rapid rate of relaxation after shortening, an increase in peak systolic intracellular calcium and a more rapid return of intracellular calcium to the diastolic level after stimulation [11]. The data on cell shortening and intracellular calcium are illustrated in the top panel of Fig. 5. This figure compares the effect of equimolar concentrations of isoproterenol (a mixed agonist) and zinterol (a β_2-selective agonist) on intracellular calcium (measured with the calcium-sensitive fluorescent dye fura-2) and cell shortening (measured as edge movement with a video edge detector) in a monolayer culture of neonatal rat ventricle cells. At the 10^{-7} M concentration employed, zinterol was approximately 40% as effective as isoproterenol in activating cAMP accumulation [11], and similarly approximately 40% as effective in enhancing the magnitude of cell shortening and peak systolic calcium. However, zinterol was equally effective as isoproterenol in enhancing relaxation (of either cell shortening or the calcium transient). In contrast to the neonatal results, zinterol at 10^{-7} M was without effect on shortening and intracellular calcium in adult rat ventricle myocytes (bottom panel of Fig. 5). Similarly, this concentration of zinterol did not result in detectable stimulation of cAMP accumulation [11].

In two separate studies, 100-fold greater concentrations of zinterol resulted in enhancement of cell shortening and peak systolic calcium in adult rat ventricle myocytes [10,11]. Interestingly, in both cases the positive inotropic response was not accompanied by enhancement of relaxation (measured as either cell shortening or intracellular calcium), despite the fact that isoproterenol-induced stimulation did have the expected relaxing effect. Kuznetsov et al. [11] also found that even this high concentration of zinterol had only a minor effect on cAMP accumulation. Xiao et al. [53], while observing significant cAMP accumulation after zinterol stimulation, found that zinterol-induced accumulation of cAMP compartmentalized differently from norepinephrine-induced accumulation. Further, they found that zinterol-induced cAMP accumulation did not correlate with the effects of zinterol on cell shortening or intracellular calcium. Thus, both studies agree that, in the adult, β_2-adrenergic stimulation exerts effects on cell shortening and intracellular calcium that are distinct from those of β_1 stimulation, and which do not correlate well with cAMP accumulation, suggesting that the β_1 and β_2 signal transduction cascades are not identical in the adult heart. A recent study [54] suggests that the discrepancy in

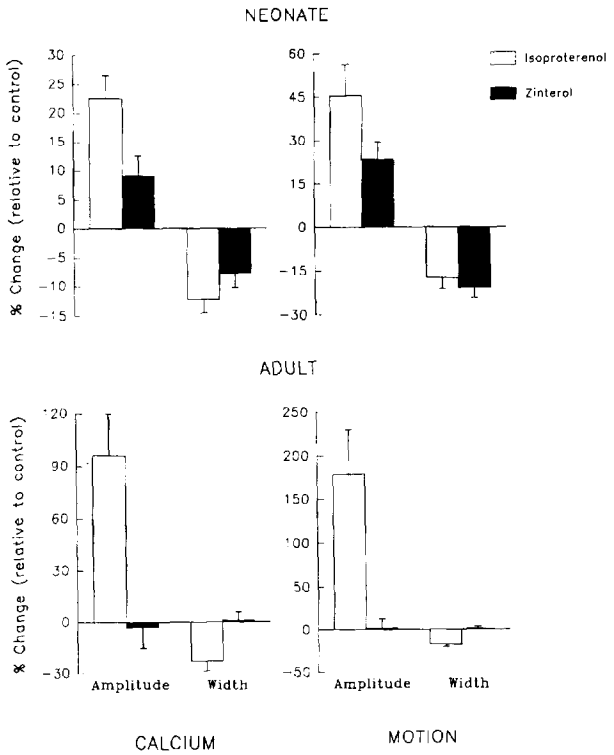

Fig. 5. Effect of isoproterenol and zinterol on cell shortening and intracellular calcium in neonatal and adult rat ventricle cells. Cells were paced at a constant frequency of 1 Hz, and superfused with buffered physiological saline at room temperature. In each case, a bolus of drug was introduced into the inlet of the superfusion chamber, to achieve the estimated final concentration of 10^{-7} M at the cell being studied. Cell shortening was recorded with a video edge detector, and intracellular calcium using the AM form of the dual excitation calcium sensitive dye fura-2 (measured as the 340/380 excitation ratio at 520 nm emission). Both agonists were effective in increasing the amplitude of cell shortening and fura-2 ratio, and narrowing the duration of both responses (i.e. enhancing relaxation) in the neonatal preparation. However, only isoproterenol was effective in the adult cells at this concentration. Results are expressed as mean ± s.e.m.; $n = 9$ and 6 for isoproterenol in the neonate and adult, respectively; $n = 11$ for zinterol in both preparations. (Reprinted with permission from Kuznetsov V, et al. Circulation Research 1995;76:40–52.)

β_1 and β_2 activation in the adult rat ventricle may arise in part from a second, inhibitory pathway activated by β_2-adrenergic agonists, and in fact demonstrated a response of adult myocytes to lower doses of zinterol when the cells were pretreated with PT. It remains to be determined if this inhibitory pathway also is functional in the neonate.

5. Muscarinic responsiveness

The most profound effects of muscarinic agonists are found in the atrium, where they activate a potassium current ($i_{K,ACh}$) via a direct G protein linkage between the M_2 receptor and channel protein, without formation of any cytosolic second messenger [55,56]. A similar mechanism exists in the sinus node, where the resulting hyperpolarization could account for the well known negative chronotropic effect of muscarinic agonists. However, it has been found that muscarinic agonists inhibit the pacemaker current i_f at much lower concentrations than those required to activate $i_{K,ACh}$, and this has been suggested to be an important contributor to the vagal control of heart rate [57]. In ventricular tissue, the effect of muscarinic agonists is most evident in the presence of simultaneous β-adrenergic stimulation, where muscarinic activation inhibits the effect of the adrenergic agent (accentuated antagonism), presumably by an action at the level of cAMP formation or breakdown.

This latter pathway has been found to vary developmentally. While much of the pioneering work in this area was done in the embryonic chick ventricle (for review see [58,59]), muscarinic receptor–effector coupling in the avian and mamalian hearts appears to differ significantly [60]. The present discussion will therefore be restricted to the mammalian literature. Osaka and colleagues [61] studied the ability of carbachol to antagonize the effect of isoproterenol on $i_{Ca,L}$ in the rabbit ventricle. They reported that while 10 μM carbachol shifted the isoproterenol dose–response relation by 2.8-fold to the right in adult ventricle cells, the same concentration completely abolished the effect of isoproterenol (up to 10 μM) in the newborn rabbit ventricle. They attributed the decreased effectiveness of muscarinic agonists in the mature ventricle to a reduced contribution of a PT-sensitive G protein toward the inhibition of adenylate cyclase.

While the M_2 receptor is the most familiar cardiac muscarinic receptor, it is not the only subtype present in the heart. Rosen et al. reported a positive chronotropic response of neonatal, but not adult, canine Purkinje fibers to acetylcholine [2]. The positive chronotropic effect was blocked by the M_1 selective antagonist pirenzepine but not by the M_2 selective antagonist AFDX-116. The effect also was abolished in PT-treated fibers, indicating that the receptor was coupled to a PT-sensitive G protein. A qualitatively similar effect also was reported in the newborn rat ventricular septum preparation [15]. Subsequent studies in cell culture confirmed the presence of a positive chronotropic muscarinic response in the newborn rat ventricle that involved an M_1 receptor and PT-sensitive G protein [9]. Further, sympathetic innervation of the ventricular cells in culture resulted in the loss of this response, although a presynaptically based response, due to the release of neuronal catecholamines via M_1 receptor activation, was observed in the innervated cultures. These results are illustrated in Fig. 6. The left panel demonstrates a positive chronotropic response in muscle cultures to the M_1-selective agonist McN A343. The β-blocker propranolol does not alter the response. The right panel shows

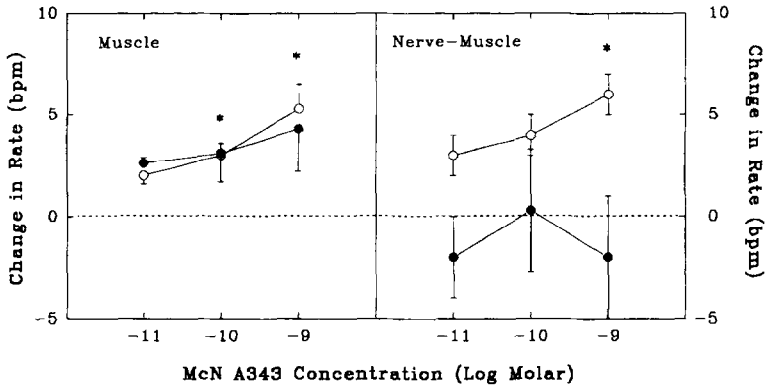

Fig. 6. Effect of the M_1-selective muscarinic agonist McN A343 on spontaneous rate in non-innervated and sympathetically innervated neonatal rat ventricle cell cultures. The left panel illustrates the positive chronotropic response of McN A343 in a non-innervated muscle culture (open circles). This effect was not prevented when the experiment was repeated in the presence of the β-blocker propranolol (filled circles). The right panel illustrates that McN A343 has a qualitatively similar positive chronotropic response in sympathetically innervated cultures (open circles). However, in this case the response is prevented in the presence of propranolol (filled circles), indicating it involves a pre-synaptic action to release neuronal catecholamine. In either preparation, the McN A343 response was blocked by the M_1-selective antagonist pirenzepine (data not shown). All experiments were conducted at 37°C in culture medium. Propranolol concentration was 10^{-7} M. Data are expressed as mean ± s.e.m., with n values of 23 and 10 in the muscle preparation in the absence and presence of propranolol, respectively, and 10 and 6 in the innervated preparation under the corresponding conditions. Data derived from Sun et al. [9].

the corresponding data for innervated cultures. In this case the response is completely lost in the presence of propranolol (or pirenzepine — data not shown), suggesting that it is entirely dependent on an M_1-mediated release of neuronal catecholamines and that the propranolol-insensitive McN A343 response is lost with innervation.

6. Summary and Conclusions

In summary, there are marked age-dependent alterations in the myocardial α_1-adrenergic, β-adrenergic and muscarinic signal transduction cascades. With maturation, an inhibitory α_1-adrenergic response appears, which differs from the pre-existing excitatory response both with respect to the specific receptor subtype involved and its G protein coupling. Neurally released NPY appears to play a critical role in regulating the expression of the inhibitory α_1-adrenergic response. Likewise, sympathetic innervation appears involved in the loss of an excitatory muscarinic response during development. Again, this response, which is M_1 mediated, differs in receptor subtype from that of the M_2 inhibitory response characteristic of the adult. Both responses are PT-sensitive, which suggests the involvement of a PT-sensitive G protein in each case, although not necessarily the identical G protein. The role of innervation in developmental regulation of the β-adrenergic response is unknown. While a distinct β_1-adrenergic response exists through development, and appears to change predominantly only with respect to magnitude, the β_2-adrenergic cascade would seem to have somewhat more complex regulation. Not only is the adult normally far less sensitive to β_2-agonists than the neonate, but the classical β-adrenergic effect to enhance relaxation along with the increase in force is absent in the adult when the β_2 (but not β_1) receptors are activated.

It is apparent from the above summary that in the case of all three autonomic receptor systems, the functional signal transduction cascades in the neonate seem designed to favor excitation (chronotropic and/or inotropic) over inhibition. The α_1-adrenergic system is exclusively excitatory in the newborn, with an opposing inhibitory cascade only becoming evident after the onset of sympathetic innervation. Similarly, prior to sympathetic innervation the muscarinic system exhibits both excitatory and inhibitory effects, with the excitatory response being lost with development. Finally, while the β-adrenergic system appears exclusively excitatory at all ages, in the neonate the β_1- and β_2-cascades both contribute to the total positive inotropic response to low concentrations of agonist, while in the adult the β_2-component only contributes at high agonist concentrations. While the reasons for the favoring of excitation cascades in the neonate is not known, it is tempting to speculate on this point. In this respect it is worth noting that in the young, increasing heart rate, rather than stroke volume, is the primary mechanism by which cardiac output is increased [62]. In this situation, excitatory autonomic mechanisms may be advantageous. Also, at the time of birth in the rat (and at other times in different species) there is a period of potential autonomic imbalance when the parasympathetic innervation to the heart is established but the sympathetic innervation is not yet well developed. During this period, having a positive chronotropic component to muscarinic action, and a positive rather than negative α_1-adrenergic response, could serve to compensate for any imbalance between the two limbs of the autonomic nervous system. Finally, while the sympathetic innervation of the heart is not fully developed at birth, there can be circulating catecholamines from the adrenal medulla, and these would be primarily epinephrine rather than norepinephrine. Since epinephrine has a much higher affinity than norepinephrine for β_2-adrenergic receptors, the presence of a strong β_2-adrenergic cascade in the neonate could be designed to respond to the circulating, rather than neuronal, catecholamines.

Lastly, one should not forget that the final physiologic response depends not only on the proximal events of receptor–effector coupling, but on more distal elements that provide the substrate for these autonomic agonists. There are significant age-dependent differences in the expression [63–65] and characteristics [66,67] of cardiac ionic currents that may serve as the ultimate targets of autonomic agonists. These developmental changes in cardiac electrophysiology are summarized elsewhere in this issue [68].

Acknowledgements

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