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THE POSTNATAL DEVELOPMENT OF ADRENOCEPTOR RESPONSES TO AGONISTS AND ELECTRICAL STIMULATION IN RAT ISOLATED ATRIA

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1 Isolated right and left atria from rats of ages ranging from newborn to adult were used to measure chronotropic and inotropic responses to noradrenaline, isoprenaline, tyramine, and electrical stimulation of intramural nerves.

2 Right atria from newborn animals showed increases in rate with noradrenaline, isoprenaline, and tyramine which did not differ significantly from those of atria from adults. The ED_{50} values for the chronotropic actions of noradrenaline and isoprenaline were not significantly different at any age from the values in adult preparations.

3 Paced left atria from newborn rats showed well developed positive inotropic responses to noradrenaline and isoprenaline. Newborn left atria (and those from 1 and 2 week old animals) were supersensitive to noradrenaline but not to isoprenaline.

4 Left atria from newborn animals showed very small inotropic responses to both tyramine and field stimulation of intramural nerves. These responses developed progressively with age over the first three weeks of life. The results are discussed with respect to the development of cardiac β-adrenoceptors and of cardiac sympathetic innervation.

Introduction

Stimulation of the sympathetic nerves to the heart in adult mammals causes an increase in heart rate (the chronotropic effect) and an increase in the force of contraction (the inotropic effect). During development the establishment of these responses requires both that the heart should be innervated by sympathetic nerves which can synthesize and release noradrenaline, and that the muscle should have receptors for noradrenaline together with the post-receptor mechanisms necessary for the chronotropic or inotropic response. It has been shown that receptor development may precede the establishment of sympathetic innervation by a considerable period (Pappano, 1976).

Studies of the development of the sympathetic nervous system by the Falck fluorescence technique for catecholamines in several species including rat (De Champlain, Olson, Malmfors & Sachs, 1970; Owman, Sjoberg & Swedin, 1971), mouse (Nanot, Le Douarin & Bermon, 1974; Mackenzie & Standen, 1976) rabbit (Friedman, Pool, Jacobowitz, Seagran & Braunwald, 1968) and lamb (Lebowitz, Novick & Rudolph, 1972) have shown that the sympathetic innervation is quite sparse at birth, and develops considerably in the early postnatal period. Similarly, biochemical studies have shown the concentration of noradrenaline in the newborn heart to be 5 to 10% of that in the adult (adult level is 0.95 ± 0.07 μg/g in rat) (Iversen, De Champlain, Glowinski & Axelrod, 1967). The uptake of labelled noradrenaline is correspondingly low, and both concentration and uptake increase progressively to adult levels over the first 4 to 5 weeks in the rat (Iversen *et al.*, 1967; Atwood & Kirshner, 1976). However, the significance of these histochemical and biochemical findings as regards the establishment of functional sympathetic transmission in the heart is not obvious since in the adult heart only a small percentage of the normal noradrenaline level need be present for a maximal response to tyramine (Crout, Muskus & Trendelenburg, 1962), whereas in the mouse vas deferens Furness, McLean & Burnstock (1970) have shown that fluorescent nerve fibres are present from 9 days after birth, but neuromuscular transmision does not appear until 18 days of age.

This paper describes experiments on the chronotro-

pic and inotropic responses of rat isolated atria taken from animals ranging in age from newborn to adult. Responses were elicited by bath-applied catecholamines in order to assess the development of, and any change in sensitivity of, the receptors in the atria. In addition the ability of any sympathetic nerves present in the heart to release endogenous noradrenaline was investigated by means of electrical stimulation and tyramine.

Methods

Wistar rats of either sex and ranging in age from newborn to adult were killed by a blow on the head, the heart was rapidly removed, and the left and right atria dissected off. In preparations from adult animals half of the left atrium was cut away to leave a single sheet of tissue. Each atrium was attached to a Perspex holder by means of a platinum wire hook. A second hook attached to a length of suture thread was used to connect the preparation to an isometric force transducer, (Endevco, RCA 5734) or a transducer based on an AME single-crystal silicon element, (Standen, 1978). The tension on the right atrium was adjusted to be as low as possible consistent with recording, the left atrium was stretched to the peak of its length-tension curve. The transducers were connected to a Devices MS pen recorder.

The atria were placed in separate 20 ml jacketed organ baths in a physiological saline containing (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $NaHCO_3$ 25, $MgSo_4$1.2, KH_2PO_4 1.2 and glucose 5.6 (pH 7.2) vigorously gassed with 95% O_2 and 5% CO_2 and maintained at 32.5°C. Atropine sulphate (5×10^{-6} m) and ascorbic acid (20 mg/l) were also added. The right atria beat spontaneously, the left atria were driven at 3.5 beats/s with just suprathreshold pulses (1 to 2 V) from a Devices isolated stimulator applied to platinum plate electrodes (5 mm apart) on either side of the tissue.

Preparations were allowed to stabilize for 1 h, after which the saline was changed and the tension readjusted. Cumulative dose-response curves were obtained for noradrenaline, isoprenaline, and tyramine. Preparations were exposed to each drug concentration for 2 min before the response was measured, which was found to be long enough for a maximal response. Under these conditions there was no detectable tachyphylactic effect seen with tyramine, though high concentrations ($>5 \times 10^{-4}$ M) often had a depressant effect (cf. Blinks, 1966). The chronotropic or inotropic response to a maximally effective tyramine concentration (10^{-5} to 10^{-4} M) was the same whether this concentration was applied alone or in a cumulative dose-response sequence. After each dose-response run the preparation was washed until the rate or force returned to its baseline value.

Two methods were used to cause release of endogenous catecholamines: (a) tyramine and (b) field stimulation of intramural nerves (Blinks, 1966) by 50 mA, 5 ms pulses 3.5/s from a Farnell stimulator. These parameters were found to be maximally effective. The effects of any acetylcholine released by field stimulation (Blinks, 1966) were blocked by the atropine present in the saline. The effects of field stimulation were also completely blocked by 10^{-6} M (−)-propranolol, showing that the increased electrical stimulation did not cause the inotropic effect by a direct effect on the cardiac muscle. Field stimulation was applied before and after each dose-response run.

Drugs used were: (−)-noradrenaline (Sigma), (−)-isoprenaline bitartrate (Sigma), atropine sulphate (Sigma), tyramine hydrochloride (Sigma), (−)-propranolol (ICI) and atenolol (ICI 66082). Catecholamine solutions were made up just before each experiment.

Results are expressed as mean ± standard error and an unpaired t test was used to compare groups.

Results

Chronotropic responses from right atria

Spontaneously beating right atrial preparations from rats of different ages were used to assess the chronotropic response to adrenoceptor agonists. The results were grouped with respect to age as follows: newborn (0 to 3 days of age), 1 week (6 to 9 days), 2 weeks (12 to 16 days), 3 weeks (19 to 24 days) and adult (>6 weeks).

Figure 1a shows the chronotropic response of right atria from newborn and adult animals to noradrenaline. It is clear that right atria from newborn animals show a large chronotropic response to noradrenaline concentration $P > 0.2$ compared with adult atria). Atria from newborn rats showed slightly lower spontaneous rates in the absence of noradrenaline (171 ± 24 beats/min) than did those from adults (235 ± 11 beats/min) and often did not beat so regularly. Right atria from newborn animals also responded as well to isoprenaline as did those from adults. Similarly preparations from rats of other ages (1, 2 and 3 weeks) showed chronotropic responses which were not significantly different from those of adult preparations ($P > 0.2$). ED_{50} values for chronotropic responses to noradrenaline and isoprenaline were also measured (Table 1). There was no significant difference between the value at any age and the adult value in the case of either agonist.

Tyramine hydrochloride was used to cause release of endogenous noradrenaline in preparations from

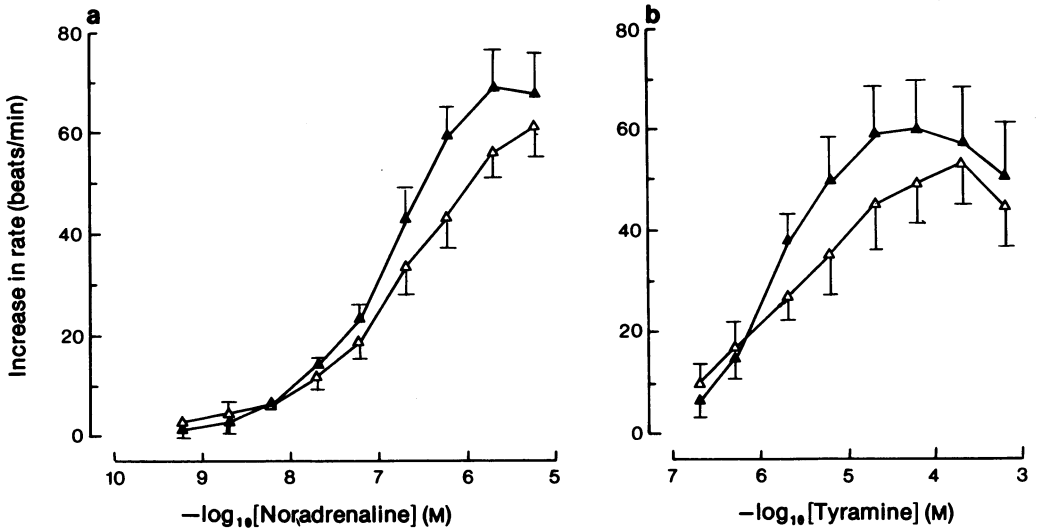

Figure 1 (a) Chronotropic responses to noradrenaline in isolated right atria. Ordinate scale: increase in rate. Abscissa scale: noradrenaline concentration. (▲) Atria from adult animals ($n = 10$); (△) atria from newborn animals ($n = 8$). The adult and newborn results are not significantly different at any noradrenaline concentration ($P > 0.2$). (b) Chronotropic responses to tyramine. Ordinate scale: increase in rate. Abscissa scale: tyramine concentration. (▲) Atria from adult animals ($n = 9$); (△) atria from newborn animals ($n = 6$). Bars give s.e. mean in each case. The adult and newborn results are not significantly different at any tyramine concentration ($P > 0.2$).

animals of different ages. The results for newborn and adult atria are shown in Figure 1b. Again the newborn preparations showed increases in rate which were not significantly different from those in adult preparations. If the maximum response (increase in rate) to tyramine was expressed as a percentage of the maximum response to noradrenaline then the value for adult preparations was 94 ± 14% and for newborn preparations 77 ± 13% (not significant, $P > 0.3$). Clearly, newborn right atrial preparations already showed good chronotropic responses to tyramine. It was not possible to obtain consistent chronotropic responses to field stimulation. This may have been because the pacing effect of field stimulation complicated the effect of the catecholomines which it released.

Inotropic responses of left atria

Dose-response curves for the positive inotropic effects of noradrenaline, isoprenaline and tyramine were obtained on paced left atrial preparations from rats of different ages. The mean dose-response relations for noradrenaline in newborn (0 to 3 day) and adult preparations are shown in Figure 2a. The maximum increase in force in the newborn preparations

Table 1 ED_{50} values for the chronotropic actions of noradrenaline and isoprenaline on isolated right atrial preparations take from rats of different ages

Age	*ED_{50} noradrenaline (M)*	*ED_{50} isoprenaline (M)*
Newborn	$1.5 \pm 0.3 \times 10^{-7}$ ($n = 8$)*	$6.6 \pm 2.9 \times 10^{-9}$ ($n = 8$)*
1 week	$2.2 \pm 0.5 \times 10^{-7}$ ($n = 9$)**	$8.2 \pm 2.0 \times 10^{-9}$ ($n = 10$)**
3 week	$1.8 \pm 0.8 \times 10^{-7}$ ($n = 5$)*	$8.1 \pm 3.0 \times 10^{-9}$ ($n = 4$)*
Adult	$1.5 \pm 0.3 \times 10^{-7}$ ($n = 10$)	$4.8 \pm 1.8 \times 10^{-9}$ ($n = 9$)

*NS ($P > 0.3$), **NS ($P > 0.2$) when compared to adult values.

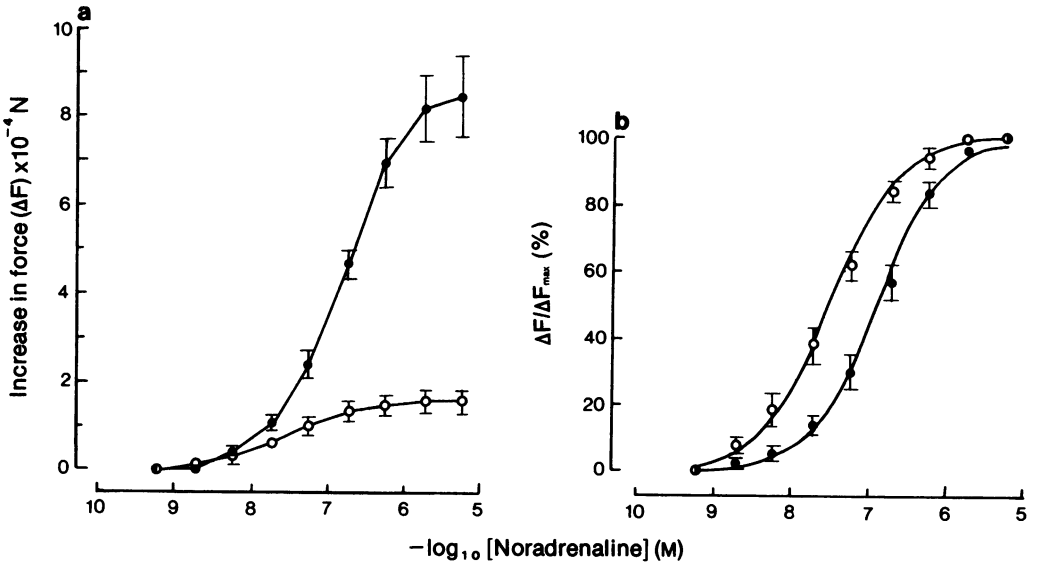

Figure 2 (a) Inotropic responses to noradrenaline in isolated left atria. Ordinate scale: increase in force. Abscissa scale: noradrenaline concentration. (●) Atria from adult animals (n = 8); (○) atria from newborn animals (n = 7). The differences in the absolute increases in force produced by these newborn and adult preparations are due to the differences in size between the atria (see text). (b) Same results as in (a) with increase in force expressed as percentage of maximum increase in force (ΔF/ΔF max). (●) Atria from adult animals; (○) atria from newborn animals. The lines are drawn assuming that one molecule of noradrenaline combines with one receptor with dissociation constants of 3.1×10^{-8} M and 1.3×10^{-7} M respectively. The vertical bars indicate s.e. means.

was about a fifth of that in the adult preparations. This difference was mainly due to the much smaller size of the newborn atria, since their baseline force (in the absence of any noradrenaline) was about one sixth of that in the adult ($2.15 \pm 0.46 \times 10^{-4}$ N and $12.65 \pm 2.31 \times 10^{-4}$ N respectively). Also, if the increases in force were expressed at $F/W^{2/3}$, where F is the increase in force, and W is the wet weight of the preparation (thus $W^{2/3}$ is proportional to area) then there was no significant difference between the maximum increases obtained in newborn and adult preparations. Thus atria from newborn rats already showed a large positive inotropic response to noradrenaline.

Figure 2a also shows that the dose-response curve from newborn atria was shifted to the left along the concentration axis when compared to that from adult preparations. This can be seen more clearly in Figure 2b, where the increases in force are expressed as percentages of the maximum increase. The ED_{50} values for the inotropic effect of noradrenaline on atria from animals of different ages are given in Table 2. In newborn atria the ED_{50} was 5 times lower than that in adult preparations, with ED_{50}s from animals

Table 2 ED_{50} values for the inotropic actions of noradrenaline and isoprenaline on isolated left atrial preparations taken from rats of different ages

Age	*ED_{50} noradrenaline (M)*	*ED_{50} isoprenaline (M)*
Newborn	$3.0 \pm 0.5 \times 10^{-8}$ (n = 7)**	$4.4 \pm 0.5 \times 10^{-9}$ (n = 7)
1 week	$8.4 \pm 2.8 \times 10^{-8}$ (n = 7)*	$6.9 \pm 2.3 \times 10^{-9}$ (n = 7)
2 week	$8.1 \pm 1.7 \times 10^{-8}$ (n = 5)*	$7.1 \pm 4.3 \times 10^{-9}$ (n = 4)
3 week	$1.09 \pm 3.10 \times 10^{-7}$ (n = 5)	$7.8 \pm 2.0 \times 10^{-9}$ (n = 4)
Adult	$1.54 \pm 0.19 \times 10^{-7}$ (n = 8)	$5.2 \pm 1.8 \times 10^{-9}$ (n = 7)

**$P < 0.001$; *$0.01 < P < 0.05$.

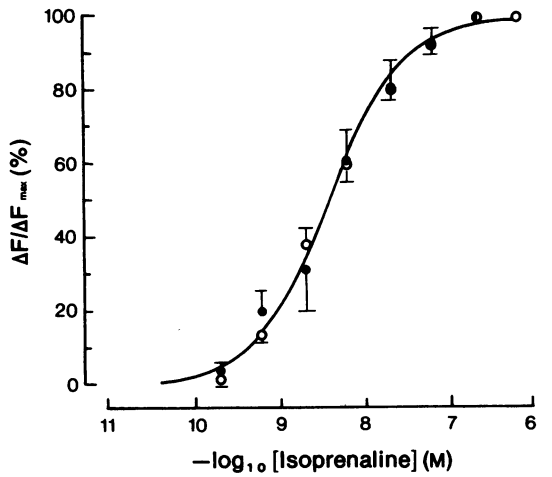

Figure 3 Inotropic responses to isoprenaline in isolated left atria. Ordinate scale: increase in force expressed as percentage of maximum increase in force. Abscisssa scale: isoprenaline concentration. (●) Atria from adult animals (n = 7); (○) atria from newborn animals (n = 7). The line is drawn assuming that one molecule of isoprenaline combines with one receptor with a dissociation constant of 4.0 × 10^{-9} M. Vertical bars give s.e. mean.

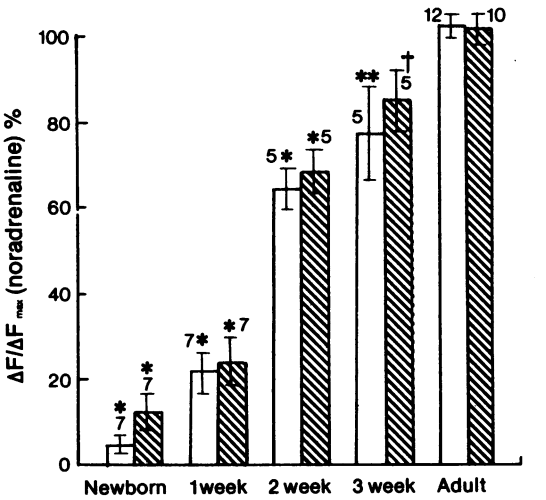

Figure 4 Inotropic responses of isolated left atria from animals of different ages to 50 mA field stimulation (open columns) and to maximally effective concentrations of tyramine (hatched columns). The responses are expressed as the increase in force as a percentage of the maximum increase in force obtained with noradrenaline. The vertical bars show s.e. means in each case, the number above each bar gives the number of experiments. *P < 0.001; **0.01 > P > 0.001; †P = 0.097 when tested against adult values.

of intermediate ages having intermediate values. Thus newborn left atrial preparations are apparently supersensitive to noradrenaline.

Isoprenaline also caused large inotropic responses in newborn preparations, but the dose-response curve was not shifted with respect to the adult curve (Figure 3), and there is no significant difference between the ED_{50} values for adult preparations and those for newborn preparations (Table 2). Responses to both noradrenaline and isoprenaline were abolished by (−)-propranolol (10^{-6} M) or by atenolol (10^{-6} M) in preparations from animals of all ages, which is consistent with the receptor being of the β-type.

Inotropic responses were also measured on the application of tyramine and of field stimulation. Dose-response curves were obtained for tyramine. Field stimulation was continued until the increase in force produced reached a maximum value. For each preparation the maximum positive inotropic effect obtained with tyramine and the maximum effect obtained with field stimulation were expressed as percentages of the maximum effect obtained with noradrenaline. The results are shown in Figure 4. In the adult preparations a maximally effective tyramine concentration (10^{-5} M to 2 × 10^{-4} M) or field stimulation caused as great an increase in force as did a maximally effective noradrenaline concentration. It can be seen that left atria from newborn rats showed very small inotropic responses to field stimulation (4.6 ± 1.9% of the increase obtainable with noradrenaline) and to tyramine (9.4 ± 4.3%). The inotropic responses to field stimulation and tyramine increased progressively with increasing age, and were close to adult values at 3 weeks. Like those to noradrenaline, the inotropic responses to tyramine or field stimulation were abolished by 10^{-6} M propranolol or atenolol.

Discussion

The large chronotropic responses seen in right atria from newborn rats show that the β-adrenoceptors and the post-receptor mechanisms necessary for chronotropic responses are already well developed at birth in the rat. This finding is in agreement with work on embryonic heart in the chick and the rat (Wildenthal, 1973; Loffelholtz & Pappano, 1974; Pappano, 1976) which shows that chronotropic effects of catecholamines can be detected before birth. Wildenthal (1973) found a small increase in rate with noradrenaline at 13 to 14 days *in utero* in rat embryo heart; the effect increased with age up until term (21 to 22

days). The present results, which show no difference in the chronotropic ED_{50} values for noradrenaline and isoprenaline between newborn and adult preparations suggest that there is no postnatal development of the β-adrenoceptors mediating chronotropic responses in the rat.

Tyramine was found to produce almost as large a chronotropic response in right atria from newborn rats as in those from adults. This is in agreement with the results of Wildenthal (1973) who found that chronotropic responsiveness to tyramine appeared just before birth in the rat. These results indicate that the rat sino-atrial node already contains sympathetic nerves capable of producing chronotropic responses at birth. The situation appears to be similar in the newborn lamb where Downing, Talner, Campbell, Halloran & Wax (1969) found that the responses to tyramine and to sympathetic nerve stimulation did not differ from those in older animals. However, in newborn dogs, Gautheir, Nadeau & De Champlain (1975) found that sympathetic stimulation caused only very small increases in heart rate, though the response had increased to adult levels at 10 days of age.

Left atrial preparations from newborn rats showed large inotropic responses to exogenous noradrenaline or isoprenaline. In the chick embryo, positive inotropic effects of catecholamines have been recorded at 4 days (Shideman, 1974; Hosey & Green, 1977; Polson, Goldberg & Shideman, 1977). It is clear that the β-adrenoceptors and mechanism for inotropic responses are already well developed at birth.

Newborn left atrial preparations were supersensitive to noradrenaline when compared to adult preparations (Table 2). This supersensitivity could be due to an alteration in the number of, or affinity of the receptors for noradrenaline, or might be due to a reduced uptake of noradrenaline into sympathetic nerve terminals since the atrial muscle is sparsely innervated at birth (De Champlain *et al.*, 1970; Owman *et al.*, 1971). No supersensitivity was seen with isoprenaline, which is not handled by the uptake mechanism (Hertting, 1964), suggesting that the apparent supersensitivity to noradrenaline was due to a reduced uptake by sympathetic nerves (Trendelenburg, 1966; Friedman, 1972).

In contrast to the responses to noradrenaline or isoprenaline, the inotropic responses to both tyramine and to electrical stimulation of intramural nerves were very small in left atrial preparations from newborn rats. The responses developed progressively with increasing age. Tyramine and electrical stimulation have been shown to release noradrenaline from sympathetic nerves by different mechanisms (Chubb, De Potter & De Schaepdryver, 1972; Thoa, Wooten, Axelrod & Kopin, 1975). The noradrenaline release which is caused by tyramine is unlike that caused by electrical stimulation in that it is independent of extracellular calcium and does not involve exocytosis. The parallel development of the inotropic responses to field stimulation and to tyramine described here suggests that these two release mechanisms develop together. This may simply be due to the ingrowth of sympathetic nerves which already possess both mechanisms. In contrast, during sympathetic nerve regeneration following denervation with 6-hydroxydopamine in the chick heart, the responses to tyramine and to field stimulation recover at quite different rates (Bennett & Malmfors, 1974).

Thus, in rat atria it appears that the β-adrenoceptors mediating both chronotropic and inotropic responses are well developed at birth. In addition, functional sympathetic innervation appears to be present as regards chronotropic responses. Functional innervation of the atrial myocardium as a whole, judged from inotropic responses, develops over the first four weeks of postnatal life. The development of sympathetic innervation indicated by histochemical and biochemical studies, then, is correlated with a functional development of inotropic responses. Histochemical studies also suggest that ventricular sympathetic innervation is completed later than that of the atria (De Champlain *et al.*, 1970; Owman *et al.*, 1971; Mackenzie & Standen, 1976) and so it seems likely that functional inotropic responses may also develop later in the ventricles than in the atria.

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