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PRESYNAPTIC AND POSTSYNAPTIC CONTRIBUTIONS TO ONTOGENY OF SYMPATHETIC CONTROL OF HEART RATE IN THE PREWEANLING RAT

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1. The dependence of heart rate on sympathetic nerve development and on ontogeny of cardiac responses to sympathetic neurotransmitters was evaluated in neonatal rats by use of the ganglionic blocking agent, chlorisondamine, and the β-adrenoceptor agonist, isoprenaline.

2 Basal heart rates of anaesthetized 4, 7 and 11 day old rats were the same as in adult rats, but the heart rate in 16 day old rats (an age at which sympathetic tone appears to be elevated) was significantly higher.

3 Chlorisondamine failed to lower heart rate significantly in 4 or 7 day old rats, but lowered the rate by 50 to 80 beats per min in the older animals and eliminated the difference in rate between 16 day old and the other ages; atropine had little or no effect on heart rate.

4 Heart rate responses to isoprenaline were lower in 4, 7 and 11 day old rats than they were in adults but were indistinguishable from them by 16 days of age, indicating that the heart is less sensitive to β-adrenoceptor stimulation early in neonatal life.

5 The ontogeny of sympathetic control of heart rate in the rat depends upon maturational changes in both presynaptic and postsynaptic elements.

Introduction

The sympathetic nervous system plays a key role in the regulation of cardiovascular function, and may even participate in normal growth and development of the heart (Claycomb, 1976; Bartolomé, Lau & Slotkin, 1977; Bareis & Slotkin, 1978). Although the ontogeny of sympathetic neuroeffector transmission has received much attention, few systematic attempts to coordinate biochemical development with physiological control of the heart have been undertaken in mammals (Pappano, 1977). Thus, while adrenergic responses in the rat heart and the development of sympathetic connections have been evaluated either biochemically or in terms of physiological effects on excised tissue, correlations of these findings with cardiac function in the intact rat have not been made. In the current study, an *in vivo* evaluation of presynaptic and postsynaptic contributions to the development of sympathetic control of heart rate in the rat has been contrasted with the biochemical and *in vitro* reports available.

Methods

Sprague-Dawley rats (Zivic-Miller) of either sex were selected at 4, 7, 11 and 16 days of age or as adults (average adult weight, 350 g) and anaesthetized with sodium pentobarbitone (25 to 50 mg/kg i.p.). Body temperature was maintained at 37°C with an infrared heating lamp controlled by a rectal thermistor, and heart rate recorded with a Model 5D Polygraph (Grass Instruments) using an ECG/Tachograph Preamplifier set to trigger on the QRS complex obtained with ECG lead II. When the heart rate became stable, the rats were given either chlorisondamine (2.1 mg/kg s.c.) or atropine (3.3 mg/kg s.c.) and the heart rate followed until it again became stable (approximately 5 to 15 min). Dose-response curves for isoprenaline were generated in chlorisondamine-pretreated rats with isoprenaline doses of 0.1 to 25 µg/kg given subcutaneously; the peak effect of each dose on heart rate and the time course of action were measured. With each dose, when heart rate had returned to the pre-isoprenaline level, a higher dose was given, the procedure repeated and a dose-response curve constructed for each rat. Repetition in any one rat of the same dose of isoprenaline gave results which were reproducible to within 10 to 15 beats per min. The entire procedure for each rat took less than 2 h from the time at which chlorisondamine was given. In some animals, sympathetic ganglionic responses were evalu-

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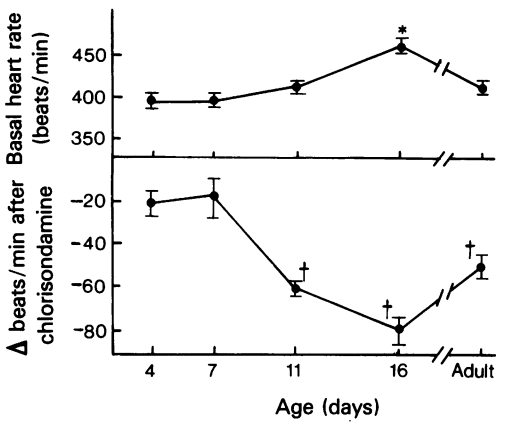

Figure 1 The effect of chlorisondamine (2.1 mg/kg s.c.) on the basal heart rate in developing and adult rats anaesthetized with pentobarbitone. *$P < 0.05$ vs. adult; †significant decrement ($P < 0.01$).

ated by atropine pretreatment followed by nicotine (0.5 mg/kg s.c.).

All drugs were dissolved in isotonic saline solution to give an injection volume of 1 ml/kg. Administration of identical volumes of saline alone produced no alterations in heart rate at any age. Doses of each drug refer to the free base.

Statistical comparisons of ED_{50} or maximal response were performed by the two-tailed, unpaired Student's *t* test. Comparisons of dose-response curves were made with the paired *t* test, with results paired by concentration. The latter test enables evaluation of whether one entire curve is different from another, as distinct from comparing individual concentration points in the unpaired *t* test. Data are reported as mean ± s.e. mean, and each age group contained 4 to 6 animals.

Drugs

Sodium pentobarbitone was obtained from Abbott Laboratories, chlorisondamine chloride from Ciba, and atropine sulphate, nicotine and (±)-isoprenaline (isoproterenol) sulphate from Sigma.

Results

After the onset of anaesthesia, the heart rate of 4, 7 and 11 day old rats was the same as in adults (385 to 410 beats per min), but the heart rate in 16 day old rats was significantly higher (Figure 1). The long-acting ganglionic blocking agent, chlorisondamine, failed to lower heart rate significantly in 4 or 7 day old rats ($P > 0.3$ and $P > 0.4$, respectively), but did lower the rate by 50 to 80 beats per min in the older animals. The effect of chlorisondamine was greatest in the 16 day old rats, and the significantly higher rate in this age group compared with the other groups was eliminated by the drug.

To ensure that chlorisondamine was indeed blocking the ganglia effectively in both young and adult animals, sympathetic ganglionic responses to nicotine were measured. Animals were pretreated with atropine to eliminate the opposing effect of parasympathetic ganglionic stimulation and then were given nicotine. In adult rats, a marked tachycardia was obtained which was totally eliminated by chlorisondamine (Table 1). The blockade persisted for at least 2 to 3 h after chlorisondamine, indicating that cardiovascular reflexes were absent during the time it took to obtain the isoprenaline dose-response curves described below. The sympathetic ganglionic response to nicotine, which was smaller in 6 to 7 day old rats than in adults, also was eliminated completely by chlorisondamine, indicating effectiveness of the ganglionic blockade in the young animals.

Isoprenaline after the chlorisondamine pretreatment was able to produce marked increases in heart rate at all ages tested (Figure 2). The dose-response curve was shifted significantly ($P < 0.02$ by paired *t* test) to the right in a parallel fashion at 4, 7 and 11 days of age compared to 16 day old rats and adults, indicating that higher doses of isoprenaline were required to produce equivalent responses in the three earliest age groups. The differences did not re-

Table 1 Effect of chlorisondamine (2.1 mg/kg s.c.) on rat heart rate response to nicotine (0.5 mg/kg s.c.)

	Δ *beats/min after nicotine*		
	No chlorisondamine	*Chlorisondamine-pretreated*	
Age		*5–15 min before nicotine*	*2–3 h before nicotine*
Adult	+123 ± 30	0 ± 0	0 ± 0
6–7 Days old	+49 ± 9	0 ± 0	—

Rats were pretreated with atropine (3.3 mg/kg s.c.) 15 min before receiving nicotine.

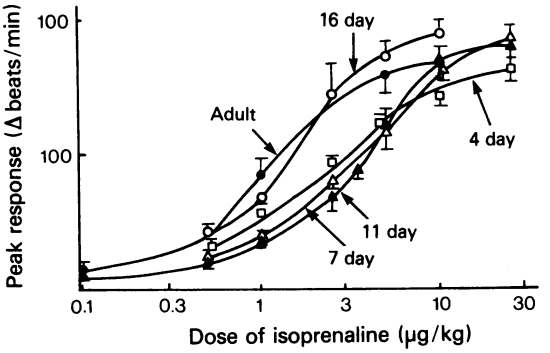

Figure 2 Isoprenaline log dose-response curves from anaesthetized developing and adult (●) rats pretreated with chlorisondamine (2.1 mg/kg s.c.). Curves from 4 (□), 7 (△) and 11 (▲) days were significantly different from that of adults ($P < 0.02$ by paired t test). (○) = 16 day old rats.

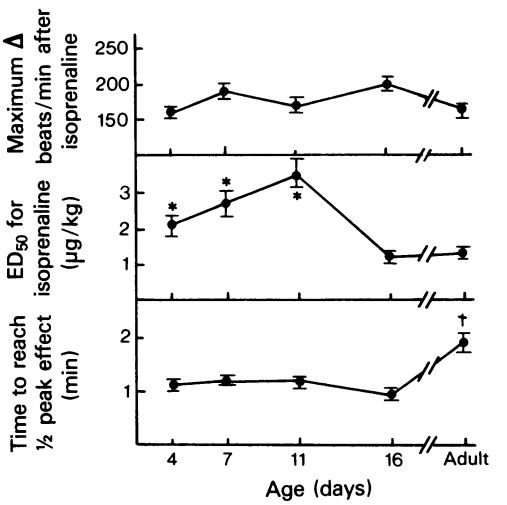

Figure 3 Maximal effect, ED_{50} and time course of onset of effect for isoprenaline in anaesthetized developing and adult rats pretreated with chlorisondamine (2.1 mg/kg s.c.). $*P < 0.05$ vs. adult; $\dagger P < 0.02$ vs. all other values.

flect a change in the maximal response, but rather a shift in the ED_{50} (Figure 3). The rate of onset of the effect of the isoprenaline injections, determined as the time required to achieve half of the peak response after each dose, was essentially the same at 4, 7 11 and 16 days of age, but was longer in adults (Figure 3).

In contrast to the effects on heart rate obtained with chlorisondamine, nicotine or isoprenaline, atropine had little or no effect at any age (Table 2), confirming results obtained previously with unanaesthetized rats (Wekstein, 1965).

Discussion

The measurements of basal heart rate and the ganglionic blockade by chlorisondamine agree well with previous inferences drawn from biochemical determinations. Two indices of sympathetic activity, the turnover of storage vesicles and the plasma activity of dopamine β-hydroxylase, indicate that sympathetic tone is elevated during the second to third weeks of postnatal age in the rat (Slotkin, 1973; Lamprecht & Wooten, 1976). In the current study, heart rates of 16 day old rats were elevated significantly compared to other ages and the elevation was eliminated by chlorisondamine, suggesting that neuronal input is responsible for the elevated heart rate. As atropine administration failed to evoke accelerated heart rates at these ages, the chlorisondamine-sensitive maturational increase with age is probably mediated through the sympathetic nervous system.

Table 2 Effect of atropine (3.3 mg/kg s.c.) on rat heart rate

Age (days)	*Δ beats/min after atropine*
4	+5 ± 4
7	+13 ± 7
11	−8 ± 7
16	−5 ± 5
Adult	+5 ± 2

Chlorisondamine did not reduce heart rate significantly in rats one week of age or younger, indicating that sympathetic input is not a contributing factor to heart rate in the early stages of postnatal development. Biochemical studies have shown that reflex sympathetic nerve stimulation of the heart can first be evoked at 6 to 8 days of age (Bartolomé *et al.*, 1977; Bareis & Slotkin, 1978; Lau & Slotkin, 1979), the age at which growth of sympathetic nerve terminals into the atrium becomes complete (Atwood & Kirshner, 1976). The reduced stimulatory effect of nicotine on heart rate in 6 to 7 day old rats is compatible with partially completed development of sympathetic connections to the heart at that age. However, the fact that tachycardia was obtained at all, indicates that pharmacological stimulation of sympathetic nerves can affect heart rate before one week of age, and consequently the absence of an effect of chlorisondamine on heart rate at 7 days probably reflects lack of tonic activity of the sympathetic system.

The development of rat cardiac β-adrenoceptors and adrenergic responses have been studied extensively in biochemical or *in vitro* physiological experiments (review, Pappano, 1977). There seems little dispute that the postsynaptic cardiac responses to catecholamines develop far earlier than does the sym-

pathetic nerve supply (Kohrman, 1973; Robkin, Shepard & Dyer, 1976; Harden, Wolfe, Sporn, Perkins & Molinoff, 1977). In the current study in which adrenergic effects on heart rate were evaluated *in vivo*, precautions were taken to ensure that the direct cardiac responses to isoprenaline was measured. Thus, autonomic reflexes were eliminated by pretreatment with chlorisondamine, and age-dependent modifications of responses due to development of sympathetic nerve reuptake mechanisms (Glowinski, Axelrod, Kopin & Wurtman, 1964) were precluded by the use of isoprenaline, a poor substrate for $Uptake_1$ (Hertting, 1964).

Four, 7 and 11 day old rats were less sensitive to isoprenaline, an effect which appeared to reflect a shift in ED_{50} but not in maximal response. The shift probably did not result from different rates of absorption, as the time courses of onset of drug action were identical in the 4, 7, 11 and 16 day old rats, despite the fact that isoprenaline responses in the 16 day olds were the same as in adults. These results suggest that, superimposed on the development of cardiac sympathetic neurones and sympathetic tone, there is an age-dependent shift in response of the heart itself to sympathetic neurotransmitters. The latter effect does not appear to be linked directly to neuronal development, as the low sensitivity to isoprenaline was still present at 11 days, a point at which cardiac sympathetic nerve supplies are fully functional (Bartolomé *et al.*, 1977; Bareis & Slotkin, 1978). Lessened response of isolated neonatal rat atria *in vitro* has been reported also (Nukari-Siltovuori, 1977), but this effect appears to be a reduction in maximal response, not in EC_{50}. It is not possible to state unequivocally whether the *in vitro* maturational difference is related to the *in vivo* responses to isoprenaline.

In conclusion, these results indicate the existence of both presynaptic and postsynaptic maturational components in the ontogeny of sympathetic neuroeffector transmission in the rat heart. Although development of functional sympathetic nerves occurs at or shortly before one week of age, there does not appear to be tonic sympathetic input to the heart until a few days later, followed by elevated sympathetic tone at two weeks of age. Independently of neuronal ontogeny, the response of the heart to isoprenaline undergoes a maturational change, with initially low sensitivity in the neonate which disappears by 16 days of age.

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References

ATWOOD, G.F. & KIRSHNER, N. (1976). Postnatal development of catecholamine uptake and storage of the newborn rat heart. *Devel. Biol.*, **49**, 532–538.

BAREIS, D.L. & SLOTKIN, T.A. (1978). Responses of heart ornithine decarboxylase and adrenal catecholamines to methadone and sympathetic stimulants in developing and adult rats. *J. Pharmac. exp. Ther.*, **205**, 164–174.

BARTOLOMÉ, J., LAU, C. & SLOTKIN, T.A. (1977). Ornithine decarboxylase in developing rat heart and brain: Role of sympathetic development for responses to autonomic stimulants and the effects of reserpine on maturation. *J. Pharmac. exp. Ther.*, **202**, 510–518.

CLAYCOMB, W.C. (1976). Biochemical aspects of cardiac muscle differentiation. Possible control of deoxyribonucleic acid synthesis and cell differentiation by adrenergic innervation and cyclic adenosine 3′:5′-monophosphate. *J. biol. Chem.*, **251**, 6082–6089.

GLOWINSKI, J., AXELROD, J., KOPIN, I.J. & WURTMAN, R.J. (1964). Physiological disposition of H^3-norepinephrine in the developing rat. *J. Pharmac. exp. Ther.*, **146**, 48–53.

HARDEN, T.K., WOLFE, B.B., SPORN, J.R., PERKINS, J.P. & MOLINOFF, P.B. (1977). Ontogeny of β-adrenergic receptors in rat cerebral cortex. *Brain Res.*, **125**, 99–108.

HERTTING, G. (1964). The fate of ^{3}H-isoproterenol in the rat. *Biochem. Pharmac.*, **13**, 1119–1128.

KOHRMAN, A.F. (1973). Patterns of development of adenyl cyclase activity and norepinephrine responsiveness in the rat. *Pediat. Res.*, **7**, 575–584.

LAMPRECHT, F. & WOOTEN, G.F. (1976). Serum dopamine β-hydroxylase activity in rat during post-natal development. *J. neural Transmission*, **39**, 301–307.

LAU, C. & SLOTKIN, T.A. (1979). Accelerated development of rat sympathetic neurotransmission caused by neonatal triiodothyronine administration. *J. Pharmac. exp. Ther.*, (in press).

NUKARI-SILTOVUORI, A. (1977). Postnatal development of adrenergic and cholinergic sensitivity in the isolated rat atria. *Experientia*, **33**, 1611–1612.

PAPPANO, A. (1977). Ontogenetic development of autonomic neuro-effector transmission and transmitter reactivity in embryonic and fetal hearts. *Pharmac. Rev.*, **29**, 3–33.

ROBKIN, M.A., SHEPARD, T.H. & DYER, D.C. (1976). Autonomic receptors of the early rat embryo heart: Growth and development. *Proc. Soc. exp. Biol. Med.*, **151**, 799–803.

SLOTKIN, T.A. (1973). Maturation of the adrenal medulla-II. Content and properties of catecholamine storage vesicles of the rat. *Biochem. Pharmac.*, **22**, 2033–2044.

WEKSTEIN, D.R. (1965). Heart rate of the preweanling rat and its autonomic control. *Am. J. Physiol.*, **208**, 1259–1262.

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