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AN ASSESSMENT OF THE EFFECTIVENESS OF NEONATAL TREATMENT WITH GUANETHIDINE AS A MEANS OF PRODUCING SYMPATHECTOMY

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1 Male Wistar rats were treated neonatally with guanethidine (following a protocol reported to produce a permanent peripheral sympathectomy) and the extent of the sympathectomy was assessed when the rats were mature.

2 The noradrenaline content of right and left atria and the ventricles was markedly reduced in 9- and 26-week old rats which had been treated with guanethidine. The extent of depletion in the ventricles was similar at both times but the atrial content increased between 9 and 26 weeks.

3 Arterial blood pressures were not significantly different in 24–26-week old conscious rats which had been treated neonatally with guanethidine compared to controls, but the baroreflex sensitivity, assessed by the lengthening of pulse interval in response to a pressor stimulus, was significantly less in the guanethidine-treated rats.

4 The blood pressure response to acute bilateral adrenalectomy was similar in control and guanethidine-treated rats.

5 Left atria and hepatic portal veins taken from 20–26-week old rats which had been treated with guanethidine showed classical signs of a denervation supersensitivity, but the chronotropic responses of right atria to noradrenergic nerve stimulation and to noradrenaline were normal.

6 Fluorescence histochemical studies on the right atria, irides and hepatic portal veins showed that the neonatal treatment had caused a variable degree of destruction both between and within animals.

7 The findings are consistent with neonatal guanethidine treatment causing extensive damage to peripheral noradrenergic nerves. However, the sympathectomy is neither complete nor permanent. In the heart, there appears to be a selective re-innervation of the atria.

8 Despite the impaired efferent sympathetic nerve activity in rats treated neonatally with guanethidine, compensatory mechanisms permit adequate cardiovascular control in the resting state.

Introduction

In 1976, Johnson and co-workers described a protocol for producing what they described as a permanent peripheral sympathectomy by the administration of guanethidine to newborn rats (Johnson, O'Brien & Werbitt, 1976). In that study, the completeness of the sympathectomy was assessed biochemically (by the measurement of tissue catecholamine levels and tyrosine hydroxylase activity in the superior cervical ganglion) and functionally (by an evaluation of the pressor responsiveness of the pithed rat preparation to sympathetic stimulation and to drugs).

Since then, several other groups of workers have used the technique of neonatal sympathectomy as a means of assessing the involvement of the sympathetic nervous system in various physiological processes. In all those studies, evaluation of the extent of the sympathectomy was based on the measurement of tissue or plasma catecholamine levels (Grzanna & Coyle, 1978; Friedman, Tassinari, Heine & Iwai, 1979; Overbeck, 1979; Levens, Peach & Carey, 1981) or the pressor responsiveness of the anaesthetized rat to noradrenaline and tyramine (Friedman *et al.*, 1979; Simon, 1981). However, there is some discrepancy in the literature regarding the degree of catecholamine depletion caused by the regime of guanethidine treatment outlined by Johnson *et al.* (1976). For instance, the levels of noradrenaline in the heart have been reported to be 0–3% (Johnson *et al.*, 1976), 2% (Friedman *et al.*, 1979), 12% (Grzanna & Coyle, 1978) or 22% (Levens *et al.*, 1981) of control levels. Blythe, Hall & Hughes (1976), using a different dose and time course of guanethidine administration, found (8 weeks after the treatment had ended) that whereas the catecholamine content of the ventricles was only

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21% of the control levels, cardiovascular control mechanisms were essentially normal. They emphasized that, although their guanethidine treatment was undoubtedly causing functional impairment of sympathetic nerves, compensatory reflex changes had occurred which led to the guanethidine administration being 'ineffective in producing a functional sympathectomy of the cardiovascular system' (Blythe *et al.*, 1976). Considering these discrepancies it seemed necessary to evaluate further the extent of the sympathectomy produced by the administration of guanethidine to newborn rats according to the protocol described by Johnson *et al.* (1976). This we have done in the present work on the basis of biochemical and histochemical techniques and functional studies in isolated cardiovascular tissues and in conscious and anaesthetized rats.

Methods

Starting 7 days after birth, male Wistar rats were treated 5 days per week for 3 weeks (total 15 injections) with guanethidine sulphate (50 mg/kg) dissolved in isotonic saline solution; the pH of the solution was adjusted to 7.4 with HCl. The drug was administered subcutaneously in a volume of 10 μl/g body weight and control animals received an equivalent volume of saline. After weaning, the rats were housed in groups of 4 in standard laboratory cages with free access to food (Pilsbury's diet, 41B) and tap water.

Blood pressure studies and baroreflex testing

Six control and 7 guanethidine-treated rats were used between 24 and 26 weeks after birth

Blood pressure recording The animals were anaesthetized with sodium methohexitone (Brietal, Lilly; 60 mg/kg i.p.) and catheters were implanted in the abdominal aorta via the caudal artery for blood pressure recording and the right jugular vein for drug administrations (full details of the catheter design and recording system have been described previously: Gardiner, Bennett & Kemp, 1980). The catheters, filled with weakly heparinized saline solution (12.5 u/ml), were fed subcutaneously (through a hollow needle) exteriorized at the back of the neck, led through a fine wire spring (attached to a harness worn by the rat) and finally out of the cage. Systolic and diastolic blood pressures were recorded on a u.v. recorder (S E 3006) using a Bell and Howell pressure transducer (Type 4-442) and an EMMA (Electromedical Multichannel Amplifier) system. The animals were allowed to recover from the anaesthesia for at least 5 h before any measurements were made. Control readings were then made for 30 min whilst the animal was quiet and undisturbed and the average pressures over that period were taken as the baseline measurements.

Baroreflex testing Baroreflex sensitivity was assessed by relating systolic blood pressure to the pulse interval of the succeeding beat (Smythe, Sleight & Pickering, 1969) during an increase in arterial blood pressure induced by infusion of methoxamine (0.4 mg/ml; 0.2 ml/min for 15 s). The slope of the line was obtained by regression analysis and was taken as an index of the baroreflex sensitivity.

Effect of adrenalectomy At the end of the experiment, the rats were anaesthetized with sodium pentobarbitone (Sagatal, May and Baker; 30 mg/kg intravenously) and the acute blood pressure responses to bilateral adrenalectomy were studied. Systolic and diastolic blood pressures were measured for 30 min pre- and post-operatively.

Organ bath studies

Six control rats and 6 guanethidine-treated rats were killed between 20 and 26 weeks after birth. Right and left atria and the hepatic portal vein were dissected free and suspended between parallel platinum wire electrodes in separate jacketed organ baths containing a physiological saline solution of the following composition (mM): NaCl 119, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4 7H_2O$ 1.2, $NaHCO_3$ 25, $NaH_2PO_4.2H_2O$ 0.9, glucose 11.1 kept at 37°C and constantly gassed with 95% O_2/5% CO_2. Atria were placed in 20 ml organ baths and hepatic portal veins in 60 ml baths. The tissues were suspended under 1 g tension which was maintained throughout the experiment. There was a 30 min equilibration period before any measurements were made. Atropine sulphate (5×10^{-6} M) was present in the baths containing the atria to abolish cholinergic responses.

Right atria Contractions of the spontaneously beating right atria were recorded through a Grass force-displacement transducer (FT 10C) attached to a Grass polygraph (model 79D) and the frequency of contractions was monitored by a tachograph (Grass model 7PF4). Only the changes in rate of the right atria were recorded since the frequency of contraction affects the force (Koch-Weser & Blinks, 1963).

Log frequency-response curves were obtained by measuring the positive chronotropic responses to noradrenergic nerve stimulation (2 ms duration, 80 V strength for 10 s) over a range of frequencies (0.1 to 10 Hz).

The positive chronotropic responses to a range of concentrations of noradrenaline ((−)-noradrenaline

bitartrate, Sigma; $1 \times 10^{-10} - 1 \times 10^{-5}$ M) were measured and log concentration-response curves constructed. Each concentration was added to the bath in a constant volume (0.2 ml) and left in contact with the tissue until the response was maximal. The bath was then rinsed twice and the tissue left to stabilize for 4 min.

Finally, log-concentration response curves to isoprenaline (1×10^{-9} M $- 1 \times 10^{-6}$ M) were obtained.

Left atria Quiescent left atria were electrically driven by field stimulation, using stimulus parameters (4 Hz; 2 ms duration; 8–10 V strength) which had little or no effect on the intramural nerves since addition of propranolol (1×10^{-6} M) and atropine (5×10^{-6} M) to the bath had no measureable effect on the force of contraction (unpublished). Contractile force was measured with a Grass force-displacement transducer as described above. Intramural noradrenergic nerves were stimulated by increasing the stimulus strength to 100 V for 10 s (Blinks, 1966). Log concentration-response curves to noradrenaline and isoprenaline were obtained in the manner described above for the right atria.

Hepatic portal veins Contractions were recorded by means of an isotonic transducer (SR1) connected to a Grass polygraph. Nerve excitation was effected by field stimulation (0.2 ms duration, 10 Hz every 4 min) using a supramaximal voltage (140 V). Log concentration-response curves to noradrenaline (3×10^{-9} M $- 3 \times 10^{-5}$ M) were obtained as before but the drug was added in a volume of 0.6 ml.

Fluorescence histochemical studies

Six control and 6 guanethidine-treated rats were killed 26 weeks after birth and the right atrium, hepatic portal vein and iris were dissected free and blotted dry. Tissues were incubated in 2% glyoxylic acid in phosphate buffer (pH 7.0) for 30 min, spread on slides, dried for 5 min, heated at 100°C for 4 min and examined under the fluorescence microscope (Furness & Costa, 1975) for the localization of noradrenergic nerves.

Tissue noradrenaline contents

Four control and 4 guanethidine-treated animals were killed at 9 and 26 weeks after birth. The right and left atria and a portion of ventricle were dissected free, blotted dry and weighed. Tissues were homogenized in 6% perchloric acid (PCA) and washed twice with 1% PCA. The noradrenaline content of the extract was analysed by high pressure liquid chromatography with electrochemical detection (Green & Macdonald, 1981).

Data analysis

Values are expressed as the mean ± 1 standard error of the mean (s.e.mean); n refers to the number of animals or preparations. Differences were tested for statistical significance using Student's unpaired t test. Log concentration-response curves and log frequency-response curves were tested for parallelism and horizontal shift by regression analysis (with 95% confidence limits).

Results

Blood pressure studies and baroreflex testing

Blood pressure recording The systolic and diastolic blood pressures of the control animals ($167 \pm 5/114 \pm 5$ mmHg; $n = 6$) were not significantly different from the pressures measured in the guanethidine-treated rats ($156 \pm 4/98 \pm 7$ mmHg; $n = 7$) although the latter were slightly lower.

Baroreflex testing Baroreflex sensitivity was significantly ($0.01 > P > 20.001$) less in the guanethidine-treated rats (0.79 ± 0.18 ms/mmHg; $n = 7$) than in the control animals (1.65 ± 0.11 mm/mmHg; $n = 6$).

Effect of adrenalectomy There was no significant difference between the systolic or diastolic blood pressures of the 2 groups under pentobarbitone anaesthesia. There was a transient insignificant increase in the systolic blood pressure of the control rats during the first 5 min after adrenalectomy which did not occur in the guanethidine-treated rats. During the following 30 min blood pressure fell in both groups to a similar extent (Figure 1).

Organ bath studies

Right atria The positive chronotropic responses to noradrenergic nerve stimulation were slightly depressed in tissues taken from guanethidine-treated rats ($n = 7$) compared to controls ($n = 6$), but the difference was not significant ($0.1 > P > 0.05$; Figure 2). Likewise, the responses to noradrenaline and to isoprenaline were not significantly different.

Left atria The positive inotropic response to noradrenergic nerve stimulation was significantly ($0.01 > P > 0.001$) less in left atria taken from guanethidine-treated rats ($3.7 \pm 0.8 \times 10^{-3}$ Newtons; $n = 7$) compared to controls ($9.0 \pm 0.8 \times 10^{-3}$ Newtons; $n = 6$). The slopes of the log concentration-response curves to noradrenaline were not significantly different in the 2 groups but there was a leftward shift in the curve obtained from the tissues taken from guanethidine-treated rats compared to

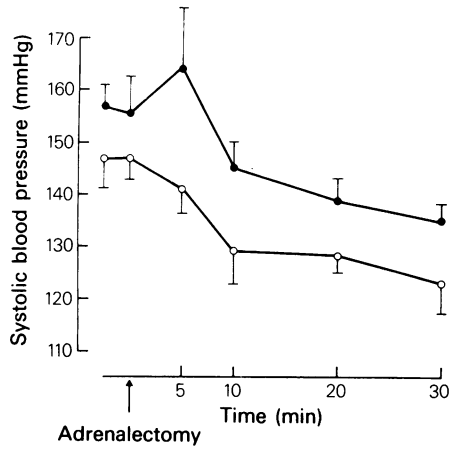

Figure 1 Systolic blood pressures (mean) before and for 30 min after bilateral adrenalectomy in pentobarbitone-anaesthetized, control (●; $n = 6$) and guanethidine-treated (○, $n = 7$) rats; vertical lines show s.e.mean.

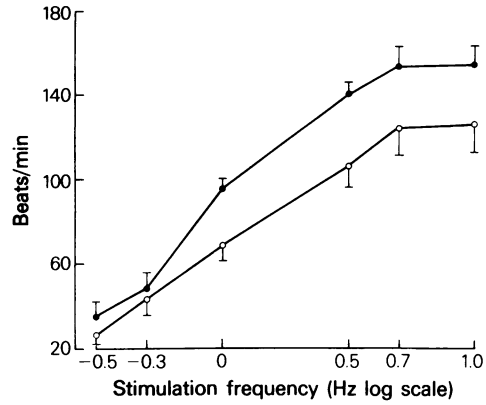

Figure 2 Chronotropic responses of spontaneously beating right atria of rat to graded increases in frequency of noradrenergic nerve stimulation. There was no significant difference between the responses obtained in atria from control (●; $n = 6$) or guanethidine-treated (○; $n = 6$) rats.

control (Figure 3); thus the sensitivity, measured as the ED_{50}, was increased 7 fold.

Hepatic portal veins The response to transmural stimulation was not significantly different in tissues taken from guanethidine-treated rats (4.1 ± 0.5 mm contraction; $n = 7$) compared to control tissues (4.7 ± 0.6 mm contraction; $n = 6$). There was a significant ($0.01 > P > 0.001$) parallel leftward shift of the log-concentration response curve to noradrenaline obtained from tissues taken from guanethidine-treated rats (Figure 4); thus the sensitivity, measured as the ED_{50}, was increased 3 fold.

Fluorescence histochemical studies

The effect of neonatal treatment with guanethidine was variable between animals and in different tissues; within the same tissue the effect was sometimes uneven. In all 6 animals studied the effects were greatest in the heart and least in the hepatic portal vein. Five of the 6 right atria examined had no noradrenergic innervation detectable by fluorescence microscopy, but the pictures in the corresponding irides ranged from a sparse to a moderate inner-

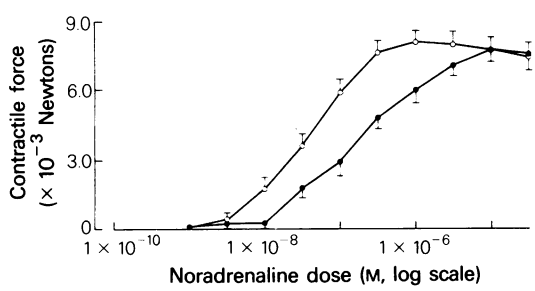

Figure 3 Log concentration-response curves for the inotropic effect of noradrenaline on electrically driven left atria from control (●; $n = 6$) and guanethidine-treated (○; $n = 6$) rats. Atria from guanethidine-treated rats showed a significant ($0.01 > P > 0.001$) parallel leftward shift of the curve, calculated from the ED_{50} (control $= 2.35 \pm 0.44 \times 10^{-7}$ M; guanethidine-treated $= 0.33 \pm 0.05 \times 10^{-7}$ M).

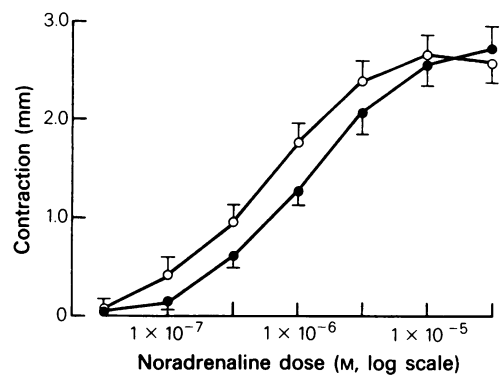

Figure 4 Log concentration-responses curves to noradrenaline obtained in hepatic portal veins from control (●; $n = 6$) and guanethidine-treated (○; $n = 6$) rats. Tissues from guanethidine-treated rats showed a significant ($0.01 > P > 0.001$) parallel leftward shift of the curve, calculated from the ED_{50} (control $= 1.12 \pm 0.14 \times 10^{-6}$ M; guanethidine-treated $= 0.47 \pm 0.09 < 10^{-6}$ M).

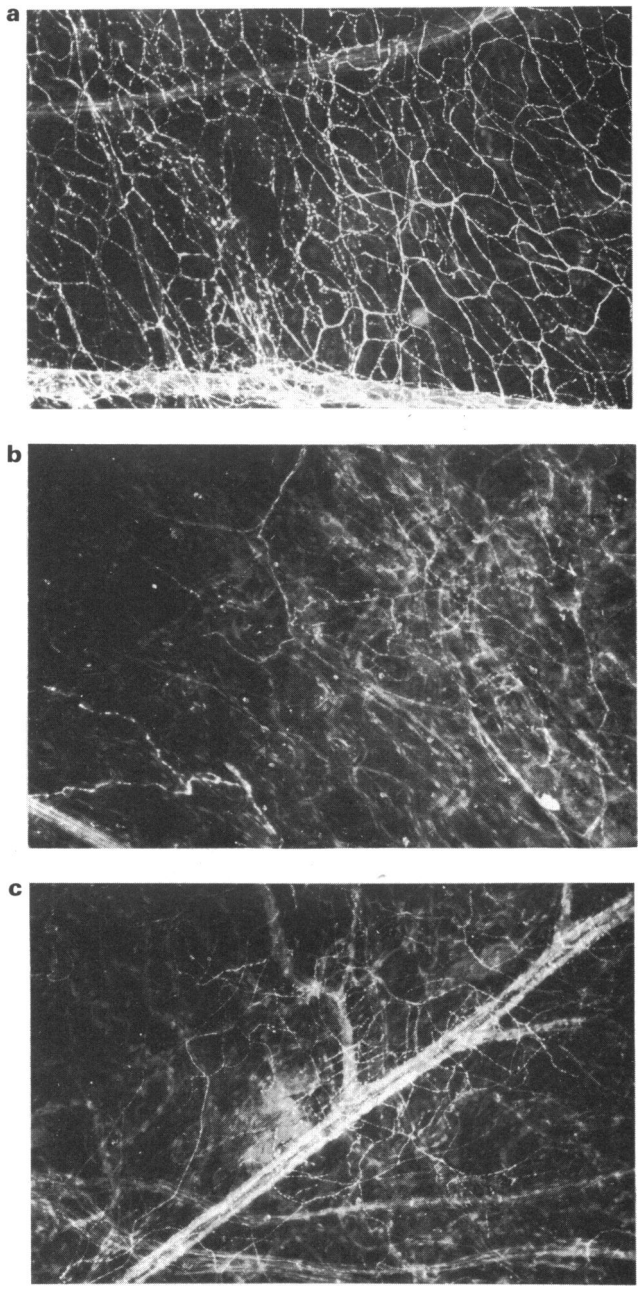

Figure 5 Stretch preparations of rat iris. (a) Preparation from a control animal. A plexus of terminal varicose fibres is present, together with bundles of non-terminal axons (running across the top of the picture. (b) Iris from a 26-week old rat treated neonatally with guanethidine. In this area terminal and non-terminal axons are sparse. (c) Another area of the same iris as in (b). In this field, terminal axons are more plentiful and non-terminal axon bundles readily detected (running across bottom of picture). Magnification ×250.

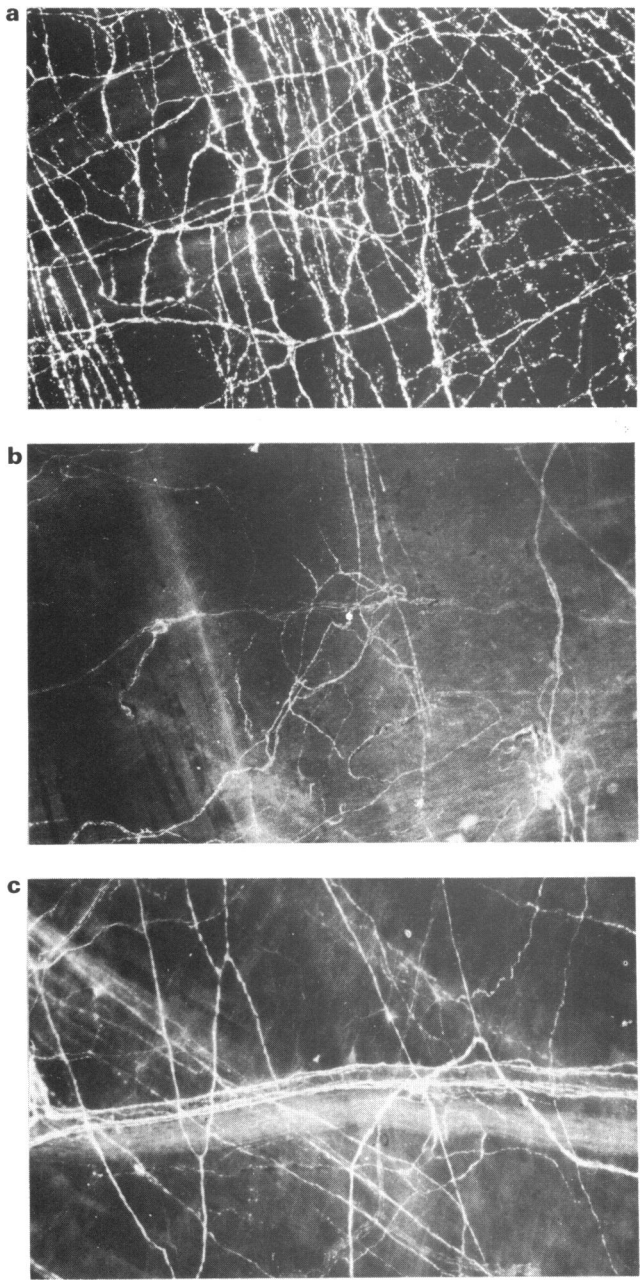

Figure 6 Stretch preparations of hepatic portal vein of rat. (a) Preparation from a control animal. Varicose nerve terminals and non-terminal axon bundles form a brightly fluorescent plexus. (b) Hepatic portal vein from a 26-week old rat treated neonatally with guanethidine. Nerve fibres are sparse in this area. (c) A different field of the same preparation as in (b). Terminal fibres and non-terminal axons are present. Note the densely innervated arteriole running across the middle of the picture. Magnification × 250.

vation. Within the same iris the picture varied in different areas (Figure 5). In all the hepatic portal veins examined there were non-terminal axon bundles present, and in the majority there were some varicose terminal fibres (Figure 6).

Tissue noradrenaline content

There was a marked reduction in the noradrenaline content of the cardiac tissues taken from the guanethidine-treated rats at both 9 and 26 weeks after birth (Table 1). The extent of depletion in the ventricular tissue was similar at both time points studied but there was some increase in the noradrenaline content of the atria between 9 and 26 weeks (Table 1).

Discussion

The present results are consistent with the earlier findings (see introduction) which showed that treatment of neonatal rats with guanethidine, following the protocol outlined by Johnson *et al.* (1976), causes a profound reduction in cardiac noradrenaline content. The extent of depletion which we measured in the ventricles is within the range reported in the literature for whole hearts (Johnson *et al.*, 1976; Grzanna & Coyle, 1978; Friedman *et al.*, 1979; Overbeck, 1979; Levens *et al.*, 1981). However, we also measured the noradrenaline content of separate right and left atria (which has not been previously done), and found some evidence for reinnervation in these tissues, since there was an increase in their noradrenaline content between 9 and 26 weeks after birth. Johnson *et al.* (1976) measured a 3% increase in the noradrenaline content of the heart between 9 and 16 weeks after neonatal sympathectomy; this they took to be insignificant, and cited the finding as evidence for the permanence and completeness of the sympathectomy. If our results are expressed as a change in the total heart noradrenaline content (taking the ventricular, right atrial and left atrial weights to be 800, 35 and 20 mg respectively, unpublished observations), then there was only a 2% increase between the 9 and 26 week old animals, but this small change obscures the marked elevation in the atrial noradrenaline contents which we measured. It is likely that the large increase of noradrenaline in the atria in the absence of any change in the ventricles reflects the route of regrowth of regenerating nerve fibres, i.e. noradrenergic nerves having to pass through the atrial tissue before being able to reach the ventricular myocardium.

Despite the marked reduction in cardiac noradrenaline content, there was very little evidence for any disturbance of resting cardiovascular status in the guanethidine-treated rats in the present study. Thus, systolic and diastolic blood pressures measured in the conscious and anaesthetized states were not significantly different. Other workers have found that arterial blood pressure is significantly lower in rats treated with guanethidine either in the conscious state (Overbeck, 1979) or when anaesthetized with pentobarbitone (Simon, 1981). However, in both those studies the adrenal glands of the guanethidine-treated animals had been demedullated. De Champlain & van Ameringen (1972) have demonstrated an important role of adrenal medullary activity in sustaining arterial pressure in animals sympathectomized with 6-hydroxydopamine (6-OHDA). Since treatment with guanethidine leaves the adrenal glands intact (Johnson *et al.*, 1976), it is possible that the systemic blood pressure in our experiments was being sustained by compensatory adrenal medullary hyperactivity. However, adrenalectomy caused a similar lowering of blood pressure in saline- and guanethidine-treated rats. This probably reflects the incompleteness of the sympathectomy in our experiments which may have resulted in less transynaptic-induction of adrenal medullary activity (Mueller, Thoenen & Axelrod, 1967) and sufficient peripheral sympathetic efferent activity to sustain the resting arterial blood pressure.

Table 1 Noradrenaline content of cardiac tissue taken from 9 and 26 week old rats treated neonatally with either guanethidine or saline

		Noradrenaline (nmol/g)		
Age	*Tissue*	*Saline*	*Guanethidine*	*% control*
9 weeks:	Ventricle	2.08 ± 0.13	0.23 ± 0.07	11%
	Right atrium	13.0 ± 1.4	1.9 ± 0.4	15%
	Left atrium	10.5 ± 0.31	1.3 ± 0.30	12%
26 weeks:	Ventricle	2.86 ± 0.14	0.28 ± 0.05	10%
	Right atrium	18.8 ± 1.50	4.8 ± 0.8	26%
	Left atrium	12.3 ± 0.21	3.3 ± 0.40	27%

Values are mean ± s.e.mean.

The pulse interval response to the drug-induced increase in arterial pressure which we measured is likely to have both vagal and sympathetic components (Coleman, 1980). Thus the reduction in baroreflex sensitivity of guanethidine-treated rats, observed in our experiments, is consistent with impaired sympathetic efferent activity. In support of this, the degree of reduction in baroreflex sensitivity was similar to that which occurs when the pulse-interval response to methoxamine is measured in the presence of propranolol (unpublished).

The changes in tissue sensitivity in the left atria and hepatic portal vein which we observed are consistent with a presynaptic denervation supersensitivity due to impairment of the neuronal re-uptake mechanisms (Trendelburg, 1966). Left atria showed these classical signs of a denervation supersensitivity whereas the right atria appeared normal, although the catecholamine contents of the two were depleted to similar extents. These findings could be explained by a selective re-innervation of the sino-atrial nodal tissue in the right atrium with a more diffuse re-innervation of the left atrium. This is supported by our fluorescence histochemical studies of the right atrium (excluding pacemaker tissue) in which there was no detectable noradrenergic innervation at a time when the noradrenaline content of the whole tissue was 26% of the control.

The present work therefore confirms earlier reports which showed that treatment of neonatal rats with guanethidine causes a marked reduction in cardiac noradrenaline content. However, our findings highlight the importance of considering specific areas and tissues with respect to patterns of regrowth, when judging the permanence of the catecholamine depletion. We have provided some evidence for impaired efferent sympathetic nerve activity in rats treated with guanethidine but, nonetheless, it appears that compensatory reflex mechanisms permit adequate cardiovascular control in the resting state. However, this is not to say that the cardiovascular system would respond to a perturbation in the normal way.

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