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An investigation of the role of adrenergic innervation in the regulation of the extraneuronal uptake of [^{3}H]-isoprenaline into rat vasa deferentia and atria

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1 Destruction of adrenergic nerves by neonatal guanethidine treatment did not affect the extraneuronal accumulation of [^{3}H]-isoprenaline by adult vasa deferentia or atria.

2 Neither pre- nor post-ganglionic denervation of vasa deferentia resulted in a significant change in extraneuronal accumulation of [^{3}H]-isoprenaline.

3 The appearance and subsequent development of extraneuronal uptake in embryonic and newborn rats did not appear to be dependent on a fully developed or functional adrenergic innervation.

4 Decreasing plasma catecholamine levels by adrenal demedullation did not have an effect on extraneuronal uptake.

5 Chronic cold exposure (7 days) significantly increased plasma levels of adrenaline, and there was a significant decrease in the corticosterone-sensitive extraneuronal uptake of [^{3}H]-isoprenaline into atria. After 14 days in the cold, plasma adrenaline levels had fallen, and were no longer significantly different from control levels.

6 The extraneuronal uptake of [^{3}H]-isoprenaline into atria after 14 days cold exposure was no longer different from that of control atria. There did not appear to be a direct correlation between plasma catecholamines and extraneuronal accumulation of [^{3}H]-isoprenaline.

7 Neither the presence of adrenergic nerves nor plasma catecholamines appear to play a major role in the regulation of extraneuronal uptake by atria and vasa deferentia of the rat.

Introduction

Many attempts have been made to explain the varying capacities of extraneuronal uptake in different tissues by relating it to some other aspect of the tissue such as the arterial supply, activity of metabolic enzymes, adrenoceptor populations and neuronal uptake (Jarrott, 1970; Gillespie & Muir, 1970; Burnstock *et al.*, 1972; Gillespie, 1976; Anning *et al.*, 1978). While no such relationships were found to exist, there is considerable evidence that a direct relationship may exist between the adrenergic innervation and extraneuronal uptake. For example, it has been shown that chemical sympathectomy using 6-hydroxydopamine (Salt & Iversen, 1973) results in a markedly reduced extraneuronal uptake. In addition, chronic pretreatment of rats with reserpine produced a decrease in the extraneuronal uptake of [^{3}H]-isoprenaline into both vasa deferentia (Morton & Mills, 1981) and atria (Morton, 1985). These latter studies showed that the change in extraneuronal uptake which follows reserpine treatment was due to a time-dependent loss of a corticosterone-sensitive component of the extraneuronal uptake. This change was not brought about by a direct effect of the drug on either the effector cell or the extraneuronal uptake mechanism. Hence, these experiments support the suggestions that extraneuronal uptake is dependent on a functional adrenergic innervation. However, since reserpine also depletes adrenergic neurones in the central system of their catecholamines, it is impossible at present to determine whether the effect of reserpine on extraneuronal uptake is a consequence of peripheral, central, or indeed a dual, action.

In this paper, the role of the peripheral adrenergic innervation in the maintenance of extraneuronal uptake is examined. Four approaches have been used. Firstly the effect of guanethidine, a peripherally acting

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pharmacological denervation agent, on extraneuronal uptake was compared with that of reserpine. Secondly, the effects of surgical denervation on uptake were investigated. Thirdly, the relationship between the embryonic and neonatal development of peripheral adrenergic innervation and of extraneuronal uptake was studied. Finally, the effects of circulating catecholamines on extraneuronal uptake were assessed.

Methods

Animals

Male albino rats (Otago Wistar) were used, weight-matched at body weights of 225–275 g. Atria and vasa deferentia were dissected from rats killed by stunning and decapitation. Each vas deferens was trimmed of its adherent fat, mesentery and blood vessel and then opened with a single longitudinal cut using very fine scissors. The epithelial lining of the lumen of each tissue was stripped from the rest of the tissue in a single movement, using watchmaker's forceps.

Uptake studies

Since isoprenaline is not a substrate for either neuronal uptake or monoamine oxidase (MAO) (Hertting, 1964; Callingham & Burgen, 1966), and further, since the major portion of non-specific binding of catecholamines appears to be associated with connective tissue and this binding is not temperature-dependent (Gillespie *et al.*, 1970), extraneuronal uptake was calculated as the difference in accumulation of [^{3}H]-isoprenaline measured at 37° and at 0°C after incubation for up to 120 min.

Tissues were incubated in a modified Liley solution containing [^{3}H]-isoprenaline 5×10^{-5} M (200 nCi ml^{-1}); catechol-*O*-methyl transferase (COMT) inhibitor, 3,5 dimethoxy 4-hydroxybenzoic acid (Nicodejevic *et al.*, 1970) 5×10^{-6} M; ascorbic acid 1.14×10^{-4} M and EDTA 2.7×10^{-5} M; bubbled with a mixture of 95% O_2 and 5% CO_2. The medium was continually replaced during incubation. In some experiments corticosterone (100 μM) was used to block the extraneuronal uptake and was present in the bathing medium throughout the experiment. Corticosterone was used at this concentration because it causes near maximal inhibition in rat heart (Salt, 1972; Uhlig *et al.*, 1976). This was confirmed in preliminary studies.

Tissues were removed from the incubation media after periods of 5, 7, 30, 60 or 90 min. They were carefully blotted, weighed and digested in 2 ml sodium hydroxide (0.5 M) for several days. After this time, a 0.8 ml aliquot of each sample was neutralized with hydrochloric acid, 9 ml of scintillant added and the radioactivity of each sample counted in a Packard Tri-Carb β-Liquid scintillation spectrometer. The amount of isoprenaline accumulated in the tissues was estimated assuming that the tritiated isoprenaline was not chemically distinct from the non-tritiated isoprenaline in the incubation media. The isoprenaline content was calculated in pmol mg^{-1} tissue wet weight.

Reserpine treatment

Intraperitoneal injections of reserpine (1 mg kg^{-1}) were given daily for 7 days.

Guanethidine treatment

Starting one week after birth, male rats were injected five days per week for three weeks with either guanethidine (50 mg kg^{-1} day^{-1} s.c.) or an equal volume of saline. Injections were made in a volume of 5–10 μl g^{-1} body weight. After weaning at 21 days after birth, these rats were housed in standard cages until they were about three months old, when the uptake studies were carried out.

Fluorescence histochemistry

The presence of adrenergic nerves was demonstrated by the visualization of noradrenaline using the glyoxylic acid technique described by de la Torre & Surgeon (1976).

Denervation of the vas deferens

Preganglionic denervation (decentralization) was carried out by the method of Sjöstrand (1962) while the animals were anaesthetized with ether. The hypogastric nerve to one vas deferens of each rat was sectioned and a 2 cm length of the nerve removed. The other hypogastric nerve was left intact and the vas deferens it innervated was used as a control. One week after surgery the animals were killed and the tissues prepared for uptake experiments as previously described.

Postganglionic denervation was performed by the method of Kasuya *et al.* (1969) while the animals were anaesthetized with ether. The hypogastric plexus was removed carefully from the base of one of the vasa deferentia. The contralateral organ served as a control. Animals were killed one week after surgery and the tissues prepared for uptake experiments as previously described.

With both procedures, denervation was considered to be complete, if electrical stimulation of the hypogastric nerve did not elicit a contraction of the vas deferens.

Adrenal demedullation

Bilateral adrenal demedullation was performed via the dorsal approach while the animals were anaesthetized with ether (Ingle & Griffith, 1962). The adrenal cortex was sliced with a scalpel and the adrenal medulla extruded as a whole, leaving most of the adrenal cortex in place. Operations were considered successful if there was no adrenaline detectable in the plasma 4 to 7 days after the operation (less than 40 pg ml^{-1}).

Cold exposure

Rats were housed individually in wire cages in an environmental room maintained at a temperature of 4 ± 2°C and a relative humidity of 84 ± 1%, and artificially illuminated with a light cycle of 12 h. All rats had free access to food and water. Rats were exposed to the cold for 7 or 14 days before experimentation.

Catecholamine analysis using high performance liquid chromatography (h.p.l.c.)

A combination of h.p.l.c. with electrochemical detection was used for the analysis of tissue and plasma catecholamines. This allowed the separation and detection of small amounts of catecholamines from small (less than 0.5 ml) plasma samples A μ-Bondapac C18 column was used for chromatographic separation, and a graphite-based electrochemical detector cell was used for the detection of catecholamines, following the technique described by Keller *et al.* (1976). The potential set across the detector was 0.75 mV. An eluent containing methanol (5%) and sodium heptane sulphonate (0.3 g l^{-1}) was used to improve the resolution of the peaks. Blood samples were collected from chronically cannulated rats and stored in the dark at −20°C until analysed. Catecholamines were extracted from plasma samples or from homogenized tissue samples using acid-washed alumina (Mefford, 1981). Detection limits for noradrenaline (NA) and adrenaline (Ad) were 20–50 pg.

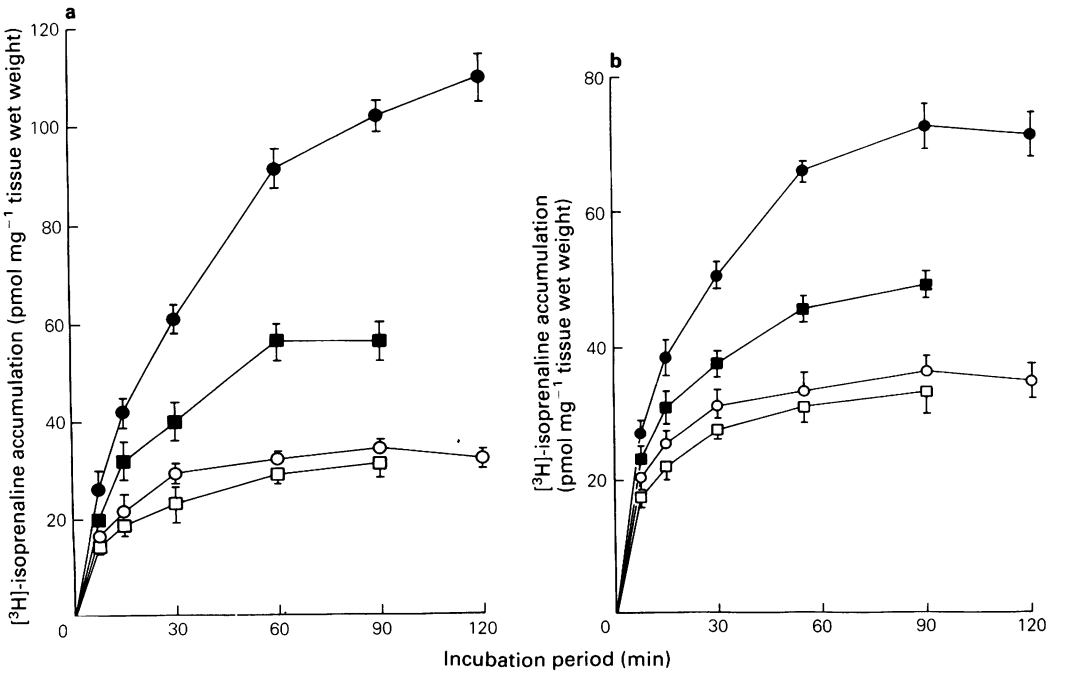

Figure 1 The time course of the accumulation of [^{3}H]-isoprenaline into atria (a) and vasa deferentia (b) measured over 120 min at 37°C (●, ■) and at 0°C (○, □). Each point represents the mean of the accumulation into 12 tissues (except after 120 min when 6 tissues were used) incubated in the absence (●, ○) or the presence (■, □) of 100 μM corticosterone. Vertical lines show s.e.mean.

Carotid cannulation

Blood samples from resting conscious rats were taken from the left carotid artery via polyethylene cannulae. Each cannula was made from a length of polythene tubing (i.d. 0.35 mm o.d. 1.05 mm) which had a small loop heat-moulded about 30 mm from the cannula tip. The cannula tip was formed by holding the tubing firmly about 15 mm from the loop and pulling rapidly to form a fine point with about half the original external diameter. The polythene tip was then cut off square about 18 mm from the 'shoulder' of the tip (i.e. where the o.d. started to decrease). Cannulae were filled with 1% heparin in saline (25 units ml^{-1}) and stoppered at the external end with a large headed pin.

For the insertion of cannulae, animals were anaesthetized with ether, and their throats shaven. The ventral aspect was displayed and a longitudinal incision made approximately 5 mm to the left of the midline. The carotid artery was exposed by blunt dissection and freed from surrounding tissue and nerve. Flow through the artery was stopped by a retractor covered with soft silastic rubber tubing inserted beneath the vessel. A ligature was tied at the dorsal end of the carotid artery to occlude the vessel and a second and third ligature were placed loosely around the proximal end and the middle of the exposed artery. The cannula was inserted through a small incision, and slowly fed into the artery and the ligatures tied, thus holding the cannula in place. The retractor was removed, and the artery returned to its normal position. The patency of the cannula was checked at this stage, before any further surgical procedures. Each functional cannula was anchored by suturing it to adjacent muscles, the cannula loop was placed under the salivary glands and the cannula threaded under the skin to exit through a small incision between the ears on the back of the head of the animal.

The patency of each cannula was checked daily and patent cannulae were refilled with heparinized saline.

Composition of Liley solution

The modified Liley solution was of the following composition (mM): Na^+ 140.4, Cl^- 132.5, K^+ 5.0, $H_2PO_3^-$ 1.0, Ca^{2+} 2.0, HCO_3^- 17.9, Mg^{2+} 1.0, glucose 11.1, pH 7.2–7.4.

Statistical analysis

Values are expressed as means ± s.e.mean. A one-way analysis of variance was performed on the data and the statistical significance was determined by use of Student's *t* test.

Table 1 Accumulation of [^{3}H]-isoprenaline into atria and vasa deferentia from control and reserpine-treated rats measured in the presence and absence of corticosterone (100 μM)

	Accumulation of [^{3}H]-isoprenaline (pmol mg^{-1} wet weight)				
Incubation period (min)	*7*	*15*	*30*	*60*	*90*
Atria					
0°C					
(a) Control	18.3 ± 1.8	20.0 ± 2.0	29.1 ± 1.8	34.2 ± 1.6	32.0 ± 1.0
(b) Reserpine	22.3 ± 2.2	23.2 ± 1.6	28.3 ± 2.3	35.2 ± 1.8	33.6 ± 1.4
(c) Reserpine/ corticosterone	20.1 ± 2.0	24.2 ± 2.1	26.1 ± 2.8	31.4 ± 2.1	30.1 ± 2.0
37°C					
(a) Control	26.0 ± 3.2	42.1 ± 2.4	60.5 ± 2.9	90.8 ± 2.6	101.6 ± 2.6
(b) Reserpine	28.0 ± 4.6	36.7 ± 3.8	40.0 ± 4.0	54.1 ± 3.8	58.2 ± 5.2
(c) Reserpine/ corticosterone	26.2 ± 4.3	38.6 ± 3.1	48.7 ± 3.9	57.6 ± 4.0	52.0 ± 4.8
Vasa deferentia					
0°C					
(a) Control	21.8 ± 2.6	25.5 ± 0.7	34.3 ± 1.0	38.5 ± 0.7	41.2 ± 1.3
(b) Reserpine	26.9 ± 4.9	28.7 ± 1.6	33.1 ± 0.8	37.4 ± 0.8	39.3 ± 0.9
(c) Reserpine/ corticosterone	20.2 ± 3.4	24.2 ± 1.3	29.4 ± 2.0	41.9 ± 1.5	38.3 ± 1.4
37°C					
(a) Control	28.5 ± 2.8	39.6 ± 2.9	51.0 ± 2.8	66.4 ± 3.0	72.5 ± 3.2
(b) Reserpine	25.2 ± 3.6	32.2 ± 3.6	43.6 ± 2.7	49.7 ± 2.5	54.8 ± 2.1
(c) Reserpine/ corticosterone	21.1 ± 4.2	30.3 ± 2.3	40.3 ± 2.1	46.2 ± 1.2	51.0 ± 1.8

Each value represents the mean ± s.e.mean of the accumulation of [^{3}H]-isoprenaline into 12 (a and b) or 6 (c) tissues.

Drugs and chemicals

Drugs and chemicals used were: (−)-isoprenaline HCl (Sigma), 3,5 dimethoxy, 4-hydroxybenzoic acid (ICN Pharmaceutical Inc.), reserpine, guanethidine (CIBA-GEIGY Ltd), heptane sulphonate (Eastman Kodak Company), alumina (BDH Chemicals Ltd,), (±)-[7-^{3}H]-isoprenaline HCl (Amersham International Ltd.).

Results

Accumulation of [^{3}H]-isoprenaline by control tissues

At 0°C the accumulation of [^{3}H]-isoprenaline by both atria and vasa deferentia from untreated rats was rapid during the first 7 min and gradually slowed over the next 20 min (Figure 1). From 30 min onwards no significant increase in [^{3}H]-isoprenaline accumulation was observed. After an incubation period of 90 min, 32.0 ± 1.0 pmol mg^{-1} tissue (wet weight) and 41.2 ± 1.3 pmol mg^{-1} tissue (wet weight) of [^{3}H]-isoprenaline had accumulated in the atria and vasa, respectively. The presence of corticosterone in the incubation media did not affect the accumulation of [^{3}H]-isoprenaline (Figure 1). At 37°C the accumulation of [^{3}H]-isoprenaline by atria and vasa was rapid over the first 7 min (Figure 1), and slower thereafter. From 60 min onwards there was no significant further increase in the accumulation of [^{3}H]-isoprenaline into vasa deferentia, while atria continued to accumulate [^{3}H]-isoprenaline over the entire 120 min of the experiment. No attempt was made to determine whether this accumulation reached a plateau, and when comparisons of the accumulations were made, the values measured at 90 min were used. At 90 min the accumulation of atria and vasa deferentia was 101.6 ± 2.6 pmol mg^{-1} tissue and 72.5 ± 3.2 pmol mg^{-1} tissue (wet weight), respectively. Differences in accumulations of [^{3}H]-isoprenaline at 37°C and 0°C were significant for vasa and atria at each time interval ($P < 0.05$ at 7 min, $P < 0.01$ from 15 min onwards).

Effects of reserpine and guanethidine on accumulation of [^{3}H]-isoprenaline

At 0°C the accumulation of [^{3}H]-isoprenaline by both atria and vasa deferentia from reserpine-treated rats in the presence or absence of corticosterone was not significantly different from control at any time over the 90 min incubation period (Table 1). At 37°C the initial accumulation of [^{3}H]-isoprenaline by tissues from reserpine-treated rats did not differ significantly from control. However, at 30 min the accumulation by both tissues from reserpine-treated rats was significantly lower than the corresponding control accumulation, and remained so at 60 and 90 min incubation. Calculated decreases in extraneuronal uptake as a result of reserpine treatment were 64% in atria and 51% in vasa. There was no difference between the uptake into atria from control or reserpine-treated rats after incubation in corticosterone at 0°C and 37°C (Table 1). There was no difference between the values for the accumulation of [^{3}H]-isoprenaline by either atria or vasa deferentia taken from guanethidine-treated, saline-treated or untreated rats, after 7 or 60 min of incubation (Figure 2).

When sections of atria and vasa deferentia from rats treated with either reserpine or guanethidine were examined by fluorescence microscopy, no intensely fluorescent neurones were seen (data not shown).

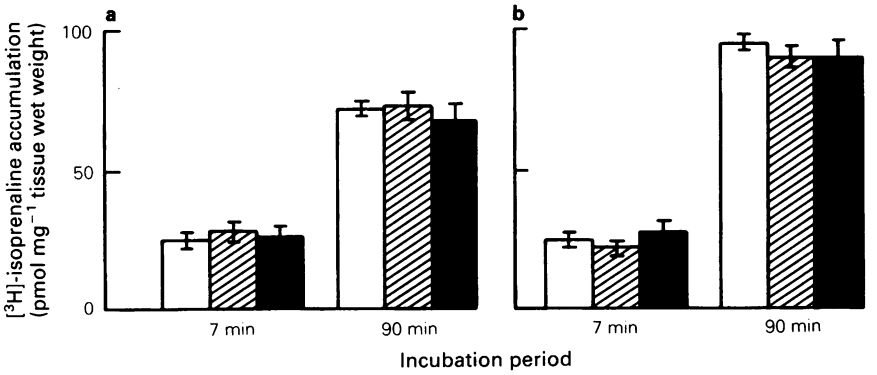

Figure 2 The accumulation of [^{3}H]-isoprenaline in (a) vasa deferentia and (b) atria from guanethidine-treated (solid columns), saline-treated control (hatched columns) and untreated control (open columns) rats at 7 or 90 min of incubation. Each column represents the mean of 10–12 tissues and vertical lines show s.e.mean.

Hence, both treatments produced extensive and comparable amine depletion..

Accumulation of [^{3}H]-isoprenaline by vasa deferentia after denervation and decentralization

Neither denervation nor decentralization had a significant effect on the accumulation of [^{3}H]-isoprenaline by vasa deferentia at 5 or 60 min of incubation at 37°C, when compared with the accumulation by unoperated control vasa deferentia from the same animals (Figure 3).

Embryonic and neonatal development of adrenergic nerves and of extraneuronal uptake

Fluorescence histochemistry studies for the demonstration of adrenergic nerves were made using hearts from rats at day 16 of gestation (d16), hearts at d20, hearts and atria at 4 days after birth (4d), and atria at 12d and 20d ($n = 3$ throughout) after birth. No fluorescent axons were observed in tissue slices prepared from hearts of embryonic rats or from the hearts of rats examined at 2d. In hearts and atria taken at 4d, faint green background fluorescence and occasional weakly fluorescent single axons were observed. These axons were usually confined to the periphery of the tissue. At 12d, the fluorescence intensity of the axons was greater than at 4d, although the distribution of the axons was still sparse and predominantly peripheral. By 21d, the characteristic innervation of the blood vessels of the atria was apparent, although the intensity of the fluorescence was lower than that usually seen in adult preparations. Nevertheless, the pattern of distribution of the axons was extensive, and similar to that seen in adults.

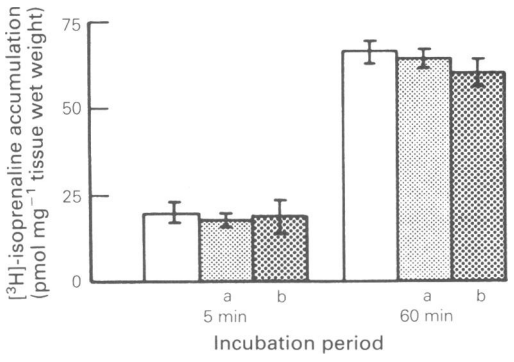

Figure 3 The accumulation of [^{3}H]-isoprenaline into unoperated (open columns), decentralized (7 day) (a) or denervated (7 day) (b) vasa deferentia. The accumulation was measured at 5 or 60 min of incubation at 37°C. Each column represents the mean of 6–12 tissues and vertical lines show s.e.mean.

The noradrenaline content of whole hearts from embryonic (d16 and d20) and neonatal (2d and 4d) rats, and of atria from neonatal (4d and 8d), young (12d, 16d and 21d) and adult (>70d) rats was analysed (Figure 4a and b). In the whole hearts of embryos, at d16 noradrenaline was detectable 20–50 pg per heart) but not in concentrations large enough to be measured accurately. By d20, a significant amount of noradrenaline was present ($0.27 \pm 0.02\,\mu g^{-1}$ tissue). The amount of noradrenaline was increased by 2d and further increased by 4d. In atria, levels of noradrenaline increased between 4d and 21d, but remained significantly lower than those measured in adult tissues (Figure 4b).

Extraneuronal accumulation of [^{3}H]-isoprenaline by hearts and atria at 90 min incubation was undetectable at d16 of gestation, but by d20 a significant extraneuronal accumulation was measured (Figure 4c). No significant increase in accumulation occurred up to 4d after birth. Atrial uptake developed rapidly between 4d and 12d (Figure 4d). From 12d after birth no further significant increase in extraneuronal uptake occurred.

Blood sampling and plasma catecholamine levels

Noradrenaline and adrenaline concentrations were measured in blood samples collected from rats during cannulation, and then 1, 3 and 7 days after cannulation (Figure 5, closed symbols). The levels of noradrenaline in plasma samples taken during cannulation were elevated significantly above those measured at any other time. By the first day after cannulation the noradrenaline level had fallen dramatically, and there was no further significant change over the next six days. On day 7, the noradrenaline concentration was $670 \pm 120\,pg\,ml^{-1}$ plasma.

Plasma levels of adrenaline were significantly elevated during the cannulation operation and on day 1 ($P < 0.01$ compared with day 7). Plasma levels of adrenaline were also significantly elevated during the cannulation operation ($P < 0.01$ compared with days 3 or 7) and declined gradually over the next 7 days. (There was no significant difference between the plasma adrenaline levels at cannulation and on day 1, on day 1 and on day 3, and on day 3 and day 7.) Catecholamine levels were measured in plasma from rats that had blood samples withdrawn only on the seventh days (Figure 5, open symbols). There was no significant difference in either noradrenaline or adrenaline levels between rats sampled once only and rats from which several samples had been taken.

The importance of the correct choice of sampling procedure is illustrated by the results obtained for the effect of reserpine on plasma catecholamine levels when two different sampling procedures were used. When blood samples were taken from decapitated

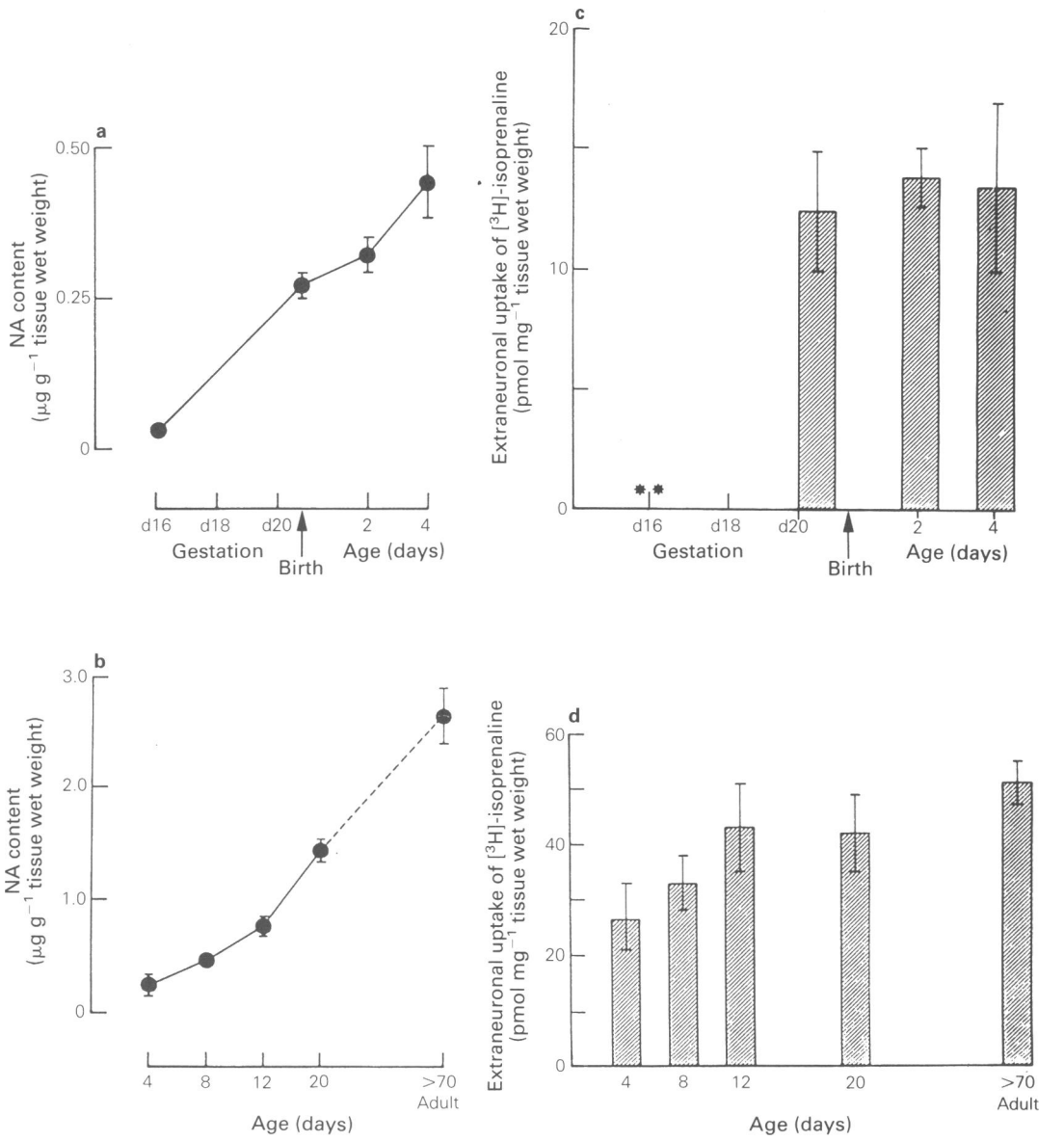

Figure 4 The noradrenaline (NA) content of (a) whole hearts from embryonic and neonatal rats and (b) atria from rats 4–20 days after birth and from adults. The value shown at d16 of gestation is the noradrenaline content from a single determination; each of the other points represents the mean of at least 5 tissues and vertical lines show s.e.mean. (c and d) The development of extraneuronal accumulation of [^{3}H]-isoprenaline at 90 min incubation into (c) whole hearts of embryonic and neonatal rats and (d) atria from rats 4–20 days old and adults. **At day 16 there was no significant difference between the accumulation at 0°C and 37°C so the uptake was not calculated. In (c) and (d) each of the other values represents the mean of the difference between the accumulations at 37°C and 0°C (n = 5 for each point at each temperature) and vertical lines show s.e.mean.

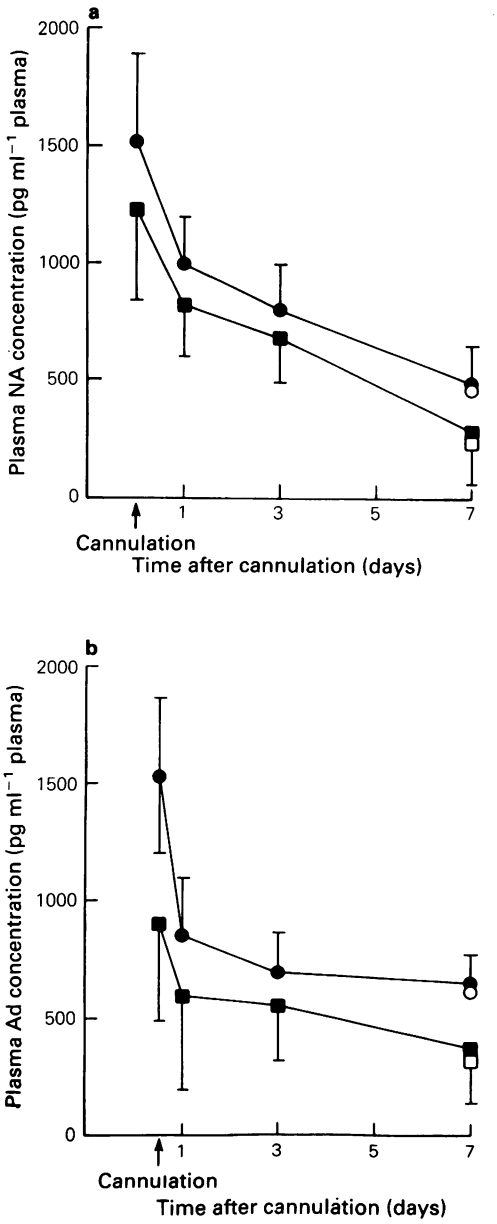

Figure 5 The effect of chronic cannulation on the noradrenaline (NA; a) and adrenaline (Ad; b) content of plasma taken from 4 control rats (●), and 4 reserpine-treated rats (■), measured over 7 days. These samples were taken from the same group of rats on successive days. The open symbols represent the noradrenaline/adrenaline content of blood taken from rats whose blood had not previously been sampled. Each point shows the mean of 4 blood samples and vertical lines represent s.e.means.

rats, plasma levels of noradrenaline were found to be significantly ($P < 0.01$) lower in rats treated with reserpine for 7 days ($450 \pm 120\,pg\,ml^{-1}$) than in control rats ($1400 \pm 120\,pg\,ml^{-1}$). On the other hand, when samples were taken via carotid cannulae, no significant difference was observed (Figure 5).

Effect of adrenal demedullation on extraneuronal accumulation of [^{3}H]-isoprenaline

Adrenal demedullation resulted in plasma levels of adrenaline of less than $50\,pg\,ml^{-1}$. However, noradrenaline levels were not significantly different from those in control plasma ($0.78 \pm 0.14\,ng\,ml^{-1}$ compared with $0.62 \pm 0.12\,ng\,ml^{-1}$). The accumulation of [^{3}H]-isoprenaline by atria and vasa deferentia was measured 7 days after adrenal demedullation (Figure 6). There was not significant effect of this procedure on accumulation by either atria or vasa deferentia after 7 or 90 min of incubation.

Effect of cold exposure on extraneuronal accumulation of [^{3}H]-isoprenaline

The effect of cold exposure of rats on the plasma levels of adrenaline and noradrenaline is illustrated in Figure 7. Cold exposure for 7 days caused a significant ($P < 0.05$) increase in adrenaline level. However, after 14 days the adrenaline level had fallen, and was not significantly different from control. The plasma level of noradrenaline was not significantly different from control at either time.

The time-course of accumulation of [^{3}H]-isoprenaline by atria from cold-exposed rats (7 days) measured at 0°C did not differ significantly from control at any time over the 90 min of the experiment (Figure 8). At 37°C, there was no significant difference between uptake into control atria, after 7 or 15 min of incubation and uptake into atria from cold-exposed rats measured at the same times. However, from 30 min onwards, there was a small (approximately 25%) but significant decrease in uptake into tissues from cold-exposed rats. After incubation of atria in the presence of corticosterone for up to 90 min at 37°C there was no significant difference between accumulation by atria from cold-exposed and control rats (Table 2).

After 14 days of cold-exposure [^{3}H]-isoprenaline accumulation by atria was no longer significantly different either from control or from accumulation by atria from rats exposed to the cold for 7 days (Figure 9).

After 7 days of cold exposure, [^{3}H]-isoprenaline accumulation by vasa deferentia, measured over a 90 min incubation period, was not significantly different from control, either at 0°C or at 37°C (Table 2).

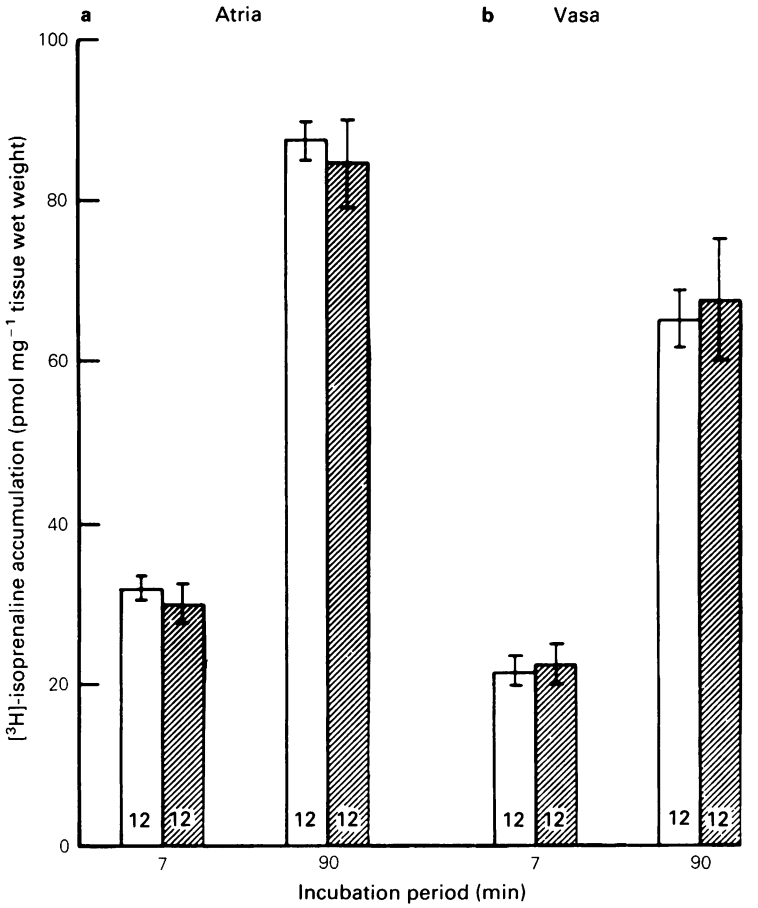

Figure 6 The extraneuronal accumulation of [^{3}H]-isoprenaline into (a) atria and (b) vasa deferentia from 7 day adrenal demedullated rats (hatched columns) and from sham-operated control rats (open columns). [^{3}H]-isoprenaline accumulation was measured after 7 or 90 min of incubation at 37°C. Each column shows the mean obtained from the number of tissues depicted at the base of each column and vertical lines represent s.e.means.

Discussion

It has been shown previously that chronic treatment of rats with reserpine results in the loss of a corticosterone-sensitive component of extraneuronal uptake (Morton, 1985). In order to provide a direct comparison with reserpine treatment, another pharmacological agent, guanethidine, was used to induce a functional adrenergic denervation. Guanethidine differs from reserpine in two important respects. Firstly, it has a cytotoxic effect on the neurones, whereas the major effect of reserpine is an irreversible inhibition of the uptake of noradrenaline into storage vesicles. Secondly, unlike reserpine, guanethidine does not deplete central catecholamines, and the rats do not manifest the centrally-mediated deleterious side-effects associated with reserpine treatment. In these studies, guanethidine was administered to neonatal rats, and their tissues were examined 7 to 8 weeks later, in order to minimize a possible direct effect of guanethidine on uptake. Unlike reserpine, guanethidine had no effect on the extraneuronal uptake into either atria or vasa deferentia. One possible explanation for this difference is that guanethidine treatment does not cause complete adrenergic destruction, and that the remaining neurones are sufficient to maintain the uptake process. Such a possibility must be recognized, since it has been demonstrated that although atria from guanethidine-treated rats display no neuronal fluorescence, the tissues still contain up to

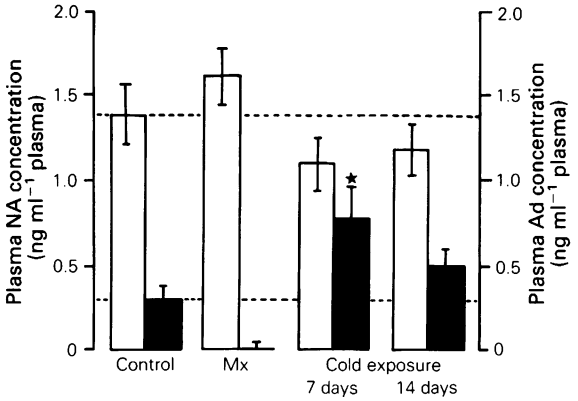

Figure 7 The effect of adrenal demedullation (Mx) or chronic cold exposure on plasma noradrenaline (NA; open columns) and adrenaline (Ad; solid columns) contents. Each column shows the mean from 5 or 6 plasma samples and vertical lines indicate s.e.means. The dotted lines show the mean level of plasma noradrenaline or adrenaline from control rats. $*P < 0.05$ when compared with the control value (one-way analysis of variance).

26% of control levels of noradrenaline (Bennett *et al.*, 1982). It has been argued that these remaining influences are sufficient to permit adequate cardiovascular control at rest (Bennett *et al.*, 1982), and the same argument could be applied to the regulation of the extraneuronal uptake. However, the fact that guanethidine lacks a central action may underlie the difference between its effects and that of reserpine.

Two methods of surgical denervation of the vasa deferentia were used, but neither resulted in a change in the extraneuronal uptake of [^{3}H]-isoprenaline. These results suggest that the functional activity of the adrenergic neurones, interrupted by both pre- and post-ganglionic denervation, does not play a major role in the control of extraneuronal uptake. This suggestion is supported by the work of Gillespie *et al.* (1970) who found no change in extraneuronal uptake in the cat after splanchnic denervation, but conflicts with the findings of Head *et al.* (1980), who found a significant decrease in the extraneuronal uptake of the denervated rabbit ear artery, and with that of Salt & Iversen (1973) who found that 6-hydroxydopamine markedly reduced extraneuronal uptake. The decrease in extraneuronal uptake observed by Salt and Iversen (1973) cannot be easily explained. However, while the action of 6-OHDA is predominantly peripheral, destruction of central adrenergic neurones has been reported (Clark *et al.*, 1972, Jacks *et al.*, 1972) and it has been noted that 6-OHDA can cross the blood brain barrier (Garver *et al.*, 1975). Thus it is possible that the decrease in extraneuronal uptake seen after 6-OHDA may not be due solely to the loss of the neurones themselves, but may be centrally mediated. It is possible that the lack of effect of surgical denervation on the extraneuronal uptake may be accounted for by the effect of remaining neurones in the tissues, and that although the tissues appear denervated, sufficient innervation remains to maintain extraneuronal uptake. However, it must be noted that treatment with both reserpine (Fleming & Trendelenburg, 1961) and guanethidine (Wigston, 1974), and surgical denervation (Kasuya *et al.*, 1969; Birmingham, 1970) all lead to the development of a state of non-specific postjunctional supersensitivity to noradrenaline. It seems unlikely that an innervation

Table 2 The extraneuronal accumulation of [^{3}H]-isoprenaline into atria and vasa deferentia from cold-exposed (7 days) rats

	[^{3}H]-isoprenaline accumulation (pmol mg^{-1} tissue wet weight)				
Incubation period (min)	*7*	*15*	*30*	*60*	*90*
Atria					
37°C					
(i)	Control/*in vitro*				
corticosterone	25.5 ± 1.4 (12)	36.3 ± 1.2 (6)	44.0 ± 2.0 (6)	58.3 ± 4.0 (6)	68.1 ± 3.8 (12)
(ii)	Cold-exposed/*in vitro*				
corticosterone	26.4 ± 2.9 (11)	34.0 ± 1.7 (6)	46.0 ± 3.7 (6)	54.7 ± 3.7 (6)	63.8 ± 3.0 (11)
Vasa deferentia					
37°C					
(i) Control	26.2 ± 2.6 (12)	36.2 ± 2.1 (6)	44.9 ± 2.1 (6)	66.6 ± 2.8 (6)	69.8 ± 3.0 (12)
(ii) Cold-exposed	24.4 ± 2.1 (11)	34.4 ± 2.8 (6)	44.0 ± 3.2 (6)	64.7 ± 2.9 (6)	67.1 ± 2.9 (12)
0°C					
(i) Control	26.2 ± 2.1 (12)	25.9 ± 1.5 (6)	32.2 ± 1.2 (6)	34.5 ± 0.5 (5)	36.2 ± 1.2 (12)
(ii) Cold-exposed	22.3 ± 2.1 (11)	25.8 ± 2.5 (5)	30.0 ± 1.0 (6)	32.8 ± 0.9 (6)	35.6 ± 1.0 (12)

Each value represents the mean ± s.e.mean of the accumulation of [^{3}H]-isoprenaline into *n* (number in parentheses) tissues.

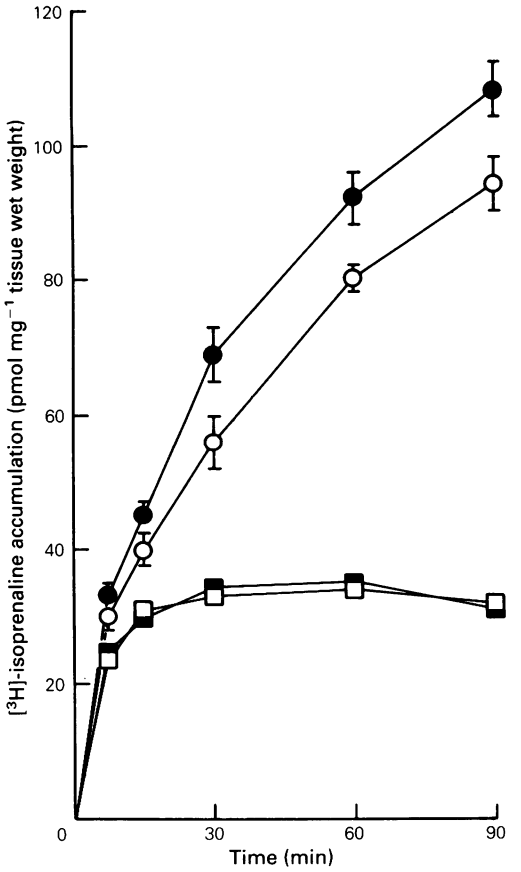

Figure 8 The effect of 7 days of cold exposure on the time course of the accumulation of [^{3}H]-isoprenaline into atria. Each point represents the mean of the accumulation into 6 to 12 tissues measured at 0°C (■, □) or at 37°C (●, ○) from control (■, ●) or cold exposed (□, ○) animals. Vertical lines show s.e.mean.

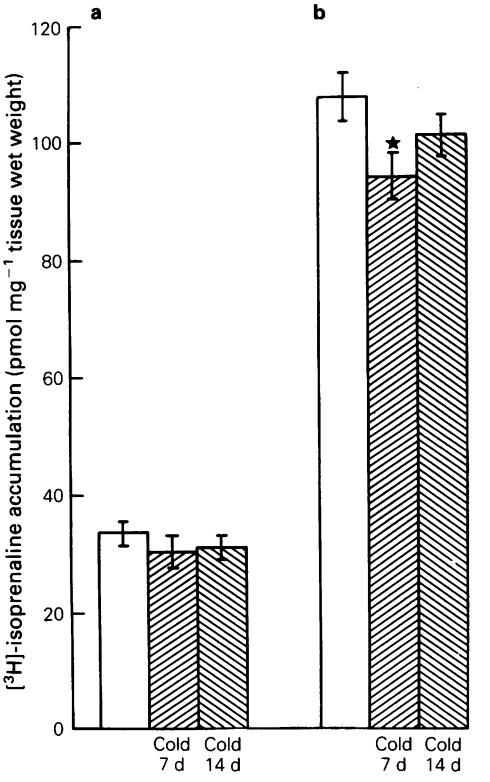

Figure 9 The effect of 7 or 14 days (d) of cold exposure (hatched columns) on the accumulation of [^{3}H]-isoprenaline measured in atria after (a) 7 or (b) 90 min of incubation at 37°C. Open columns represent accumulation in control atria. Each column represents the mean accumulation from 12 tissues and vertical lines show s.e.mean. $*P < 0.05$ when compared with the control value (one-way analysis of variance).

remaining after guanethidine-treatment or denervation would continue to maintain the extraneuronal uptake while allowing other postjunctional denervation changes to occur similar to those seen after reserpine treatment.

The role of adrenergic neurones in the regulation of extraneuronal uptake during development was also examined. The extent of the adrenergic innervation was estimated by measuring the amount of noradrenaline in the tissues (Iversen *et al.*, 1967), and by examining the tissues using fluorescence histochemistry. The results obtained in this study confirm those of Schiebler & Heen (1968) who observed no major differences in neuronal fluorescence between tissues from 1 day and 12 day old rats, and those of de Champlain *et al.* (1970) who found that the adrenergic nerves developed in the first three weeks after birth. The noradrenaline content of the tissues measured here increased markedly from late gestation to adulthood. In atria, the level of noradrenaline rose dramatically between 4 and 21 days after birth. However, by 21 days, the level of noradrenaline in the atria was still much lower than that in adult atria, although the rate at which the noradrenaline content of the atria increased, as a percentage of the level measured at 21 days is comparable with the values found by Iversen *et al.* (1967). This indicates that the transmitter storage processes are still maturing at this age, and supports the idea that for at least the first three weeks after birth the adrenergic nervous system is in a state of dynamic maturation (de Champlain *et al.*, 1970).

The fragility of the embryonic heart prevented the measurement of extraneuronal uptake at d16 of gestation, but by d20 there was a significant extraneuronal uptake, which had increased further by 2 days after birth. These results suggest that extraneuronal uptake develops with the muscle cells, and that in the first few days after birth the capacity for uptake by an extraneuronal component increases dramatically. It is somewhat surprising that there was no significant difference between extraneuronal uptake at 2 days and 4 days after birth. However, at 4d whole hearts are at the upper limit of size for *in vitro* incubation studies, and it is possible that diffusion into these tissues was inadequate, thus giving a falsely low value for extraneuronal uptake. There was a rapid increase in extraneuronal uptake between 4 days and 8 days after birth, but there was no significant difference between uptake into atria from rats at 8 days and at 12 days after birth, nor was there a difference between uptake into atria from 12 day old, 20 day old and adult rats. Thus extraneuronal uptake appears to be fully developed by 12 days, before the maturation of the adrenergic neurone appears to be complete. It appears that the presence of adrenergic neurones is not of great importance in the early expression of extraneuronal uptake. This is seen clearly in the denervation experiments and the study using rats treated neonatally with guanethidine, and implied by the studies using embryonic and neonatal rats, since extraneuronal uptake appears to develop independently of the full expression of the adrenergic nerves.

The decrease in extraneuronal uptake seen after reserpine treatment therefore cannot be explained simply by the loss of the direct influence of a functional adrenergic innervation, despite the fact that neurotransmission is severely impaired. However, the possibility remains that the extraneuronal uptake may depend, at least in part, upon circulating catecholamine levels. Although reserpine does not block the synthetic pathway for catecholamines directly, up to 70% of circulating catecholamines are purported to be derived from adrenergic neurones (Callingham & Barrand, 1979). Therefore, a significant reduction in circulating catecholamines might be expected after reserpine treatment. If the extraneuronal uptake is dependent upon the level of catecholamines as well as a local effect of the neurone, then a reduction in circulating catecholamines after reserpine treatment would result in the observed decrease in extraneuronal uptake. A role for circulating catecholamines in the regulation of extraneuronal uptake may help to account for the lack of effect of other treatments, such as denervation, on extraneuronal uptake. The limited extent of the surgical denervation used in the present work would not be expected to alter circulating catecholamine levels significantly and it is possible that the circulating catecholamines remaining after denervation may be sufficient to prevent a change in extraneuronal uptake.

In order to investigate the dependence of extraneuronal uptake on the levels of circulating catecholamines, attempts were made to both lower (by adrenal demedullation) and raise (by chronic cold exposure) these levels. Adrenal demedullation caused a fall in the plasma concentration of adrenaline to less than 50 pg ml^{-1}, but did not have a significant effect on extraneuronal uptake into either atria or vasa. Since the adrenal medulla contributes only a proportion of the total circulating catecholamines, it may be argued that circulating catecholamine levels were not lowered sufficiently to induce a change in extraneuronal uptake. However, the lack of correlation between plasma adrenaline level and extraneuronal uptake indicates that there is no direct relationship between the two.

It has been found previously (Leduc, 1961a,b; Benedict *et al.*, 1979) that chronic cold exposure results in a rapid (24 h) increase in noradrenaline excretion. Noradrenaline excretion then declines slowly, although it remains elevated after one month. Adrenaline excretion increases gradually, to reach a maximum 6–8 days after the beginning of cold exposure, and decreases rapidly thereafter (Leduc, 1961b). The elevated plasma levels of adrenaline observed in this study are consistent with an activation of the sympathetic system. However, noradrenaline levels were not markedly elevated. This was rather surprising, considering the reported increases in catecholamine turnover following cold exposure (Johnson & Pritzker, 1966; Bralet *et al.*, 1972). Nevertheless, there was a significant overall increase in the concentration of total catecholamines after cold exposure.

In experiments involving cold exposure, an inverse correlation was obtained between plasma adrenaline levels and extraneuronal uptake into atria. However, no such correlation was observed when plasma adrenaline concentration was reduced by adrenal demedullation. Furthermore, reserpine treatment, which caused a significant reduction in extraneuronal uptake, did not significantly affect plasma catecholamine levels. Consequently, it is unlikely that circulating catecholamine levels are directly responsible for the control of extraneuronal uptake.

Thus, neither the presence of functional adrenergic innervation nor the influence of plasma catecholamines appears to play a major role in the regulation of extraneuronal uptake by atria and vasa deferentia of the rat.

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