Br. J. Pharmac. (1985), **86**, 287–295

The effect of reserpine treatment on the extraneuronal uptake of [^{3}H]-isoprenaline into rat atria

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1 Treatment of rats with reserpine (1 mg kg^{-1} day^{-1}) for up to 7 days resulted in a marked decrease in a corticosterone-sensitive component of the extraneuronal accumulation of [^{3}H]-isoprenaline into their atria.

2 The change in extraneuronal uptake did not appear to be due to a direct action of reserpine on the uptake mechanism, since it was several days before the treatment had a significant effect on the accumulation of [^{3}H]-isoprenaline. Further, reserpine *in vitro* did not inhibit extraneuronal uptake.

3 The reserpine-induced change in the accumulation of [^{3}H]-isoprenaline was not an artifact due to changes in water balance, ion distribution, extracellular space or tissue atrophy. Nor was the change due to an increase in the efflux of [^{3}H]-isoprenaline from the tissue.

4 These experiments support the suggestion that the extraneuronal uptake is dependent upon a functional adrenergic innervation.

Introduction

The extent to which extraneuronal uptake is developed varies markedly between tissues as well as between species (Jarrott, 1970; Gillespie & Muir, 1970; Burnstock *et al.*, 1972; Anning *et al.*, 1978). Many attempts have been made to explain these differences by correlating them with another property of the tissue, such as its arterial supply, or the density of the α- and β-receptors. However, there is little evidence to support either of these possibilities (Gillespie, 1976). Another suggestion was that an inverse relationship exists between the extraneuronal uptake and the density of innervation. In tissues with dense innervation most of the catecholamine inactivation would be handled by the neuronal uptake and conversion by monoamine oxidase (MAO). Conversely in tissues with a poor adrenergic innervation there would be less inactivation by neuronal mechanisms and a well developed extraneuronal uptake mechanism would handle the inactivation of catecholamines (Lightman & Iversen, 1969; Gillespie & Muir, 1970). Although no such relationship was shown to exist between the density of adrenergic innervation and the extraneuronal uptake capacity (Gillespie, 1976), such findings do not exclude the possibility that a direct relationship exists between the adrenergic nerves and extraneuronal uptake.

There are several studies in which adrenergic denervation has been shown to have an effect on the extraneuronal uptake. Iversen (1965) examined the effect of immunosympathectomy and, finding that uptake$_2$ was (also) greatly reduced, concluded that uptake$_2$ was associated with postganglionic sympathetic fibres. It is now accepted that extraneuronal uptake is located extraneuronally (Clarke & Jones, 1969; Hellman *et al.*, 1971; Trendelenburg, 1980). However, the extraneuronal location of uptake$_2$ does not account for the decrease in uptake seen by Iversen in 1965. In a later study, using 6-hydroxydopamine, Salt & Iversen (1973) found that chemical sympathectomy also markedly lowered uptake$_2$. Unpublished studies by Geffen (personal communication) showed that pharmacological denervation using 6-hydroxydopamine resulted in a significant decrease in the accumulation of [^{3}H]-isoprenaline into rabbit ear artery. Our preliminary studies using reserpine as a means of pharmacological denervation showed that chronic pretreatment with reserpine (7 days) resulted in a decrease in the extraneuronal uptake of [^{3}H]-isoprenaline into the vas deferens of the rat (Morton & Mills, 1981). From these studies it was suggested that the long term regulation of extraneuronal uptake in adrenergically innervated tissues may be dependent on an intact nerve supply. The chronic administration of reserpine is a simple and effective method of sympathetic denervation; however, several findings suggest

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that reserpine treatment may also affect non-neuronal effector tissues. In this paper, the studies examining the effect of reserpine on the extraneuronal uptake have been extended to include the rat atrium, and the effect of a number of factors related to reserpine treatment which may have contributed to the reserpine-induced changes in extraneuronal uptake have been investigated.

Methods

Animals

Albino rats (Otago Wistar) were used, weight-matched at body weights of 225–275 g. Atria were dissected from the hearts of rats killed by stunning and decapitation.

Uptake studies

Since isoprenaline is a substrate for neither neuronal uptake nor for MAO (Hertting, 1964; Callingham & Burgen, 1966), extraneuronal uptake was calculated as the difference in accumulation of [^{3}H]-isoprenaline measured at 37°C and that measured at 0°C, after incubation for up to 120 min. Atria were incubated in 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, 20 mg l^{-1}; EDTA 10 mg l^{-1}; bubbled with a mixture of oxygen and carbon dioxide (95:5). The medium was continually replaced during incubation. In some experiments cocaine (100 μM) or corticosterone (100 μM) was used to block neuronal or extraneuronal uptake respectively, with cocaine or corticosterone present in the bathing medium throughout the experiment.

Calculation of isoprenaline accumulation

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 media. The isoprenaline content was calculated in pmol mg^{-1} tissue wet weight.

Efflux studies

Efflux curves were constructed by plotting the efflux of radioactivity from a tissue preloaded with [^{3}H]-isoprenaline, against time. Studies were made of the efflux from atria which had been equilibrated and then incubated for 90 min, following the usual procedure for uptake experiments. After incubation, the tissues were transferred to a small volume water-jacketed organ bath, thermostatically maintained at 37°C or 0°C, containing 1 ml of equilibration medium continuously bubbled with O_2 and CO_2 (95:5). After 30 s the bathing medium was removed via a 2 ml syringe attached to the outlet, and immediately replaced with 1 ml of fresh solution from a syringe attached to the inlet of the heat-exchange coil leading to the bath. This withdrawal and replacement procedure continued every 30 s for the first 5 min, then every min for the next 25 min. Two tissues were processed simultaneously in individual organ baths. Each ml of withdrawn solution was placed in a scintillation vial, 9 ml of scintillant added to each vial, and the radioactivity of the sample counted in a scintillation spectrophotometer. Efflux curves were constructed by plotting the radioactivity of each sample against time. An interactive computer programme was used to calculate the exponential regression equations from the data, and the half-time of the efflux of [^{3}H]-isoprenaline from the tissues was derived from these equations (a digital PDP-11 Minc was used for this analysis).

Tissue water and electrolyte content

The analysis of water and electrolytes followed the method described by Little (1964). Electrolytes were extracted into nitric acid and the concentrations determined using an EEL flame photometer. All Na^+ and K^+ contents were calculated as mmol kg^{-1} tissue dry weight and cellular concentrations of Na^+, K^+ were calculated as mmol kg^{-1} intracellular water.

Extracellular space determination

The extracellular space was calculated as that space occupied by [^{14}C]-inulin after *in vitro* incubation of tissues in modified Liley solution containing [^{14}C]-inulin (McIver & Macknight, 1974). Tissues were dissected and trimmed and then equilibrated in modified Liley solution in either a jacketed organ bath at 37°C or in a beaker on ice at 0°C, for 30 min. The equilibration medium was then replaced by the incubation medium, Liley solution containing [^{14}C]-inulin (25 nCi ml^{-1}), and the tissues incubated at 37°C or 0°C for periods up to 90 min. After incubation the tissues were dried and the inulin extracted in nitric acid (0.1 M) overnight. After extraction 2 ml aliquots of the sample and 1 ml aliquots of the medium suitably diluted with nitric acid were taken for analysis. Radioactivity was measured in a liquid scintillation spectrophotometer in a volume of 20 ml. Calculation of extracellular space was made as below.

(i) Extracellular space as a percentage of the wet weight of the tissue.

$$\text{Extracellular space} = \frac{\text{concentration of } [^{14}C]\text{-inulin from the tissue per mg wet wt}}{\text{concentration of } [^{14}C]\text{-inulin in media}} \times \frac{100}{1}$$

(ii) Extracellular space expressed as a percentage of total tissue water.

$$\text{Extracellular space} = \frac{\text{concentration of } [^{14}C]\text{-inulin in tissue water}}{\text{concentration of } [^{14}C]\text{-inulin in media}} \times \frac{100}{1}$$

Injection programme

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

Fluorescence histochemistry

The presence of adrenergic nerves in atria was demonstrated by the visualization of noradrenaline using the glyoxylic acid technique described by De La Torre & Surgeon (1976).

Fasting procedure

Two groups of rats were weight-matched and housed under identical conditions. Both groups had free access to drinking water. One group was deprived of its normal pellet diet until the average weight of the group had dropped to 75% of that of the control group. At this point both groups were killed and their tissues used for uptake studies.

Composition of Liley solution

The modified Liley solution was of the following composition (mM): Na^+ 140.4, Cl^- 132.5, K^+ 5.0, $H_2PO_4^-$ 1.0, Ca^{2+} 2.0, HCO_3^- 17.9, Mg^{2-} 1.0, glucose 11.1. The pH of the Liley solution was 7.2–7.4

Statistics

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

Drugs

Drugs used were (−)-isoprenaline HCl (Sigma); 3,5,dimethoxy,4-hydroxybenzoic acid (ICI Pharmaceuticals Inc.); reserpine (Ciba-Geigy Ltd); (±)-[7-^{3}H]-isoprenaline HCl, 5–15 Ci $mmol^{-1}$ (Amersham International); [^{14}C]-ouabain, 10–20 Ci $mmol^{-1}$ (New England Nuclear).

Results

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

At 0°C the accumulation of [^{3}H]-isoprenaline into tissues from untreated rats was rapid during the first 7 min, and then gradually slowed over the next 20 min (Figure 1). From 30 min onwards, no further significant increases in [^{3}H]-isoprenaline accumulation were observed. After a 90 min incubation period, 34.2 ± 0.5 pmol mg^{-1} tissue of [^{3}H]-isoprenaline had accumulated in the atria. The accumulation at 0°C was assumed to represent (i) [^{3}H]-isoprenaline equilibrated with the extracellular space, and (ii) non-specific binding of [^{3}H]-isoprenaline. At 37°C the accumulation of [^{3}H]-isoprenaline into atria increased rapidly over the first 7 min. After this the rate of accumulation of [^{3}H]-isoprenaline slowed, although the atria continued to accumulate [^{3}H]-isoprenaline over the entire 120 min of the experiments. No attempt was made to

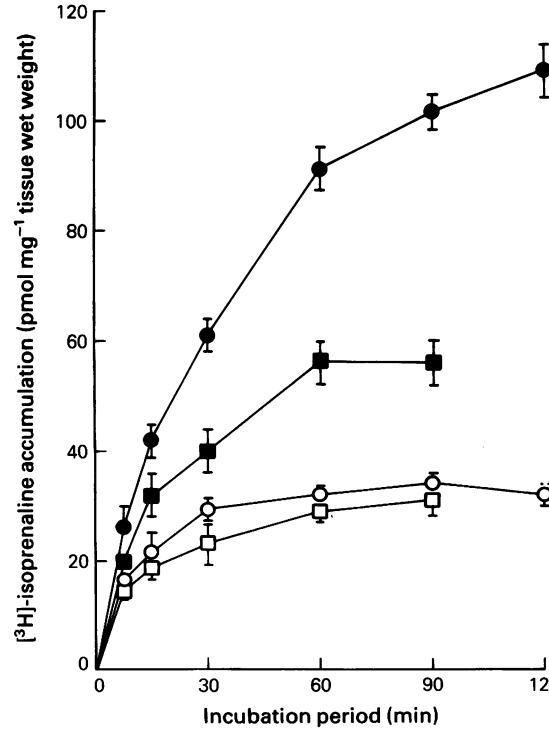

Figure 1 The time course of accumulation of [^{3}H]-isoprenaline into atria measured over 120 min at 37°C (solid symbols) and at 0°C (open symbols). Each point represents the means and vertical lines s.e.mean of the accumulation into 12 tissues except at 120 min where 6 tissues were used. The experiments were conducted in the presence (■, □) or the absence (●, ○) of 100 μM corticosterone.

Table 1 The time course for the accumulation of [^{3}H]-isoprenaline into atria from control and reserpine-treated rats

Incubation period (min)	*Accumulation of [^{3}H]-isoprenaline* (pmol mg^{-1} tissue wet weight) 7	15	30	60	90
(a) *37°C*					
Control (untreated)	26.0 ± 3.2(12)	42.1 ± 2.4(12)	60.5 ± 2.9(12)	90.8 ± 2.6(12)	101.6 ± 2.6(12)
Reserpine-treated	28.0 ± 4.6(12)	36.7 ± 3.8(12)	40.0 ± 4.0(12)**	54.1 ± 3.8(12)**	58.2 ± 5.2(12)**
Reserpine-treated/ corticosterone *in vitro*	26.2 ± 4.3 (6)	38.6 ± 3.1 (6)	48.7 ± 3.9 (6)**	57.6 ± 4.0 (6)**	52.0 ± 4.8 (6)**
(b) *0°C*					
Control (untreated)	18.3 ± 1.8(12)	20.0 ± 2.0(12)	29.1 ± 1.8(12)	34.2 ± 1.6(12)	32.0 ± 1.0(12)
Reserpine-treated	22.3 ± 2.2(12)	23.2 ± 1.6(12)	28.3 ± 2.3(12)	35.2 ± 1.8(12)	33.6 ± 1.4(12)
Reserpine treated/ corticosterone *in vitro*	20.1 ± 2.0 (6)	24.2 ± 2.1 (6)	26.1 ± 2.8 (6)	31.4 ± 2.1 (6)	30.1 ± 2.0 (6)

Measurements were made at 0°C and at 37°C. In one group, the tissues from reserpine-treated rats were incubated in the presence of 100 μM corticosterone. Each value represents the mean ± s.e.mean of the accumulation into the number of tissues shown in parentheses.
*$P<0.05$, **$P<0.01$ when compared with the corresponding control value.

see whether this accumulation reached a plateau, and when comparisons of the accumulation in atria were made, the values measured after 90 min were used. In control tissues this was 101.6 ± 2.6 pmol mg^{-1} tissue of [^{3}H]-isoprenaline. The accumulation at 37°C was assumed to represent (i) [^{3}H]-isoprenaline equilibrated in the extracellular space, (ii) non-specific binding of [^{3}H]-isoprenaline plus any temperature-dependent non-specific binding and (iii) extraneuronal uptake.

When the accumulations at 37°C and 0°C were compared, a significant difference could be measured at each time interval (at 7 min, $P<0.05$, and from 15 min onwards, $P<0.01$). 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), the extraneuronal uptake in these experiments was calculated as the difference between the accumulation of [^{3}H]-isoprenaline at 37°C and that measured at 0°C. From the above data it was calculated that at 7 and 90 min the uptake into atria was 8.5 ± 4.2 and 67.4 ± 2.6 pmol mg^{-1} tissue. The rate of uptake of [^{3}H]-isoprenaline calculated over the first 7 min was 1.20 pmol mg^{-1} tissue min^{-1}.

Accumulation of [^{3}H]-isoprenaline into atria from reserpine-treated rats

The accumulation measured at 0°C into the tissues from reserpine-treated rats was not significantly different from that measured into the tissues from control rats at any time over the 90 min incubation period (Table 1). At 37°C the initial accumulation of [^{3}H]-isoprenaline into tissues from treated rats did not differ significantly from the accumulation into tissues from control rats at 37°C, although the uptake into the tissues from reserpine-treated rats was lower than into control tissues. However, after 30 min the accumulation of [^{3}H]-isoprenaline into the tissues from reserpine-treated rats was significantly lower than the corresponding control value ($P<0.01$), and remained so after 60 and 90 min of incubation (Table 1). The accumulation of [^{3}H]-isoprenaline did not stop, but the rate of accumulation was slower than in control tissues. In reserpine-treated tissues the accumulation of [^{3}H]-isoprenaline at 37°C was significantly greater than that measured at 0°C after 30, 60 and 90 min of incubation ($P<0.01$). When the extraneuronal uptake of [^{3}H]-isoprenaline was calculated, it showed that reserpine treatment resulted in a decrease in uptake. After 90 min of incubation, the extraneuronal uptake into the atria had fallen to 42% of that in the control tissues.

Corticosterone-sensitivity of the extraneuronal uptake after reserpine treatment

In control tissues (see Figure 1), neither the time course nor the level of accumulation of [^{3}H]-isoprenaline measured at 0°C was significantly affected by the presence of corticosterone in the incubation media. At 37°C, the accumulation of [^{3}H]-isoprenaline into atria was markedly decreased after incubation in corticosterone. This decrease was statistically significant after 15 min of incubation ($P<0.05$), and onwards (after 30, 60, 90 min of incubation, $P<0.01$). After 90 min of incubation the atria incubated in the presence of corticosterone had accumulated 51.0 ± 4.0 pmol

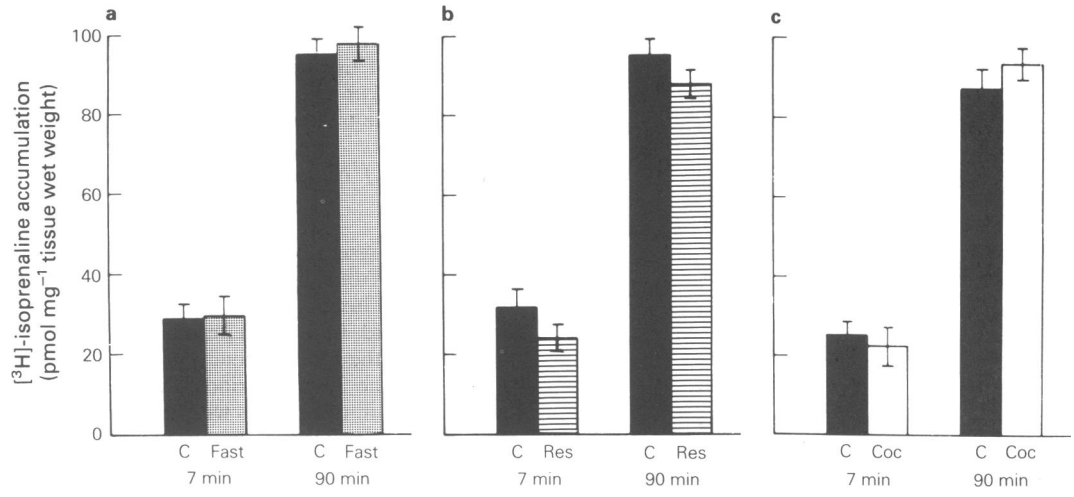

Figure 2 The accumulation of [^{3}H]-isoprenaline into atria from control rats (C; solid columns) compared with that into atria from fasted rats (Fast) (a), or atria incubated in the presence of reserpine (Res; 1 mg l^{-1}) (b), or 100 μM cocaine (Coc) (c). Each column represents the mean, and vertical lines s.e.mean, of the accumulation into 6 tissues, after 7 or 90 min of incubation.

mg^{-1} tissue. The accumulation by tissues from reserpine-treated rats of [^{3}H]-isoprenaline is included in Table 1. The accumulation was measured at both 37°C and at 0°C, and showed that inclusion of corticosterone in the incubation medium of reserpine-treated tissues failed to further decrease the extraneuronal accumulation by a significant amount.

The effect of body weight loss on the accumulation of [^{3}H]-isoprenaline.

After 7 days of reserpine treatment, the body weights of rats were typically 70–80% of the control animal weights. The weights of atria from reserpine-treated rats were 90–95% of those of the control rats. The accumulation of [^{3}H]-isoprenaline was measured at 37°C after 7 and 90 min (Figure 2a). There was no significant difference between the accumulation of [^{3}H]-isoprenaline into the tissues from fasted rats and that of the controls at either 7 or 90 min.

The effect of in vitro *reserpine on the accumulation of [^{3}H]-isoprenaline*

The accumulation of [^{3}H]-isoprenaline was measured at 37°C after 7 and 90 min of incubation, with reserpine present in the medium (1 mg l^{-1}) throughout the experiment. There was no significant difference between the uptake of [^{3}H]-isoprenaline into tissues measured in the presence of reserpine and that measured in the absence of reserpine (Figure 2b).

The effect of in vitro *cocaine on the extraneuronal uptake*

The accumulation of [^{3}H]-isoprenaline was measured with cocaine present in the medium throughout the experiment. Cocaine had no effect on the accumulation of [^{3}H]-isoprenaline into atria (Figure 2c).

Extracellular space after reserpine treatment

Measurement of the extracellular space after 90 min equilibration in [^{14}C]-inulin showed that there was no significant difference between the extracellular space of tissues from control animals (24.20 ± 0.94, $n = 6$, and 32.08 ± 1.14, $n = 6$, as a percentage of tissue wet weight and tissue water respectively), and that from reserpine-treated animals (24.34 ± 1.23, $n = 6$, and 33.58 ± 2.06, $n = 6$, as a percentage of tissue wet weight and tissue water respectively).

The effect of reserpine on tissue water and electrolytes

There was no apparent dehydration of the tissues after reserpine treatment (water content was 76 ± 1% ($n = 12$) and 74 ± 1% ($n = 12$) tissue wet weight in control and reserpine-treated tissues respectively). Similarly, reserpine had no significant effect on the Na^+ or K^+ content of the atria. The Na^+ and K^+ contents of the tissues were 98.77 ± 7.77 ($n = 6$) and 258.37 ± 114.85 ($n = 6$) mmol kg^{-1} dry weight respectively in control tissues, and 88.38 ± 11.17 ($n = 6$) and

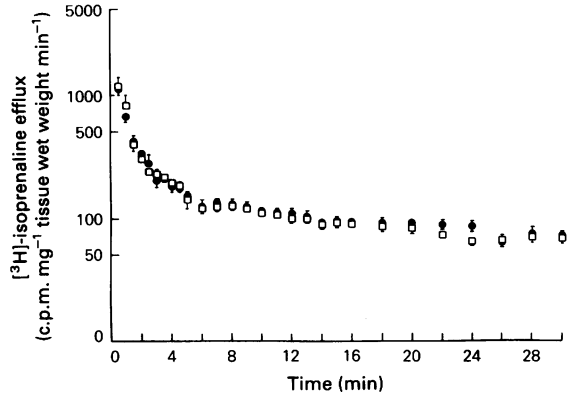

Figure 3 The time course of the efflux of [^{3}H]-isoprenaline from the atria of untreated (control) and reserpine-treated (7 days) rats. Each point represents the means, and vertical lines s.e.mean, of the rate of release of [^{3}H]-isoprenaline from 6 atria. (●) control; (□) reserpine-treated.

245.88 ± 11.75 ($n = 6$) mmol kg^{-1} dry weight respectively in reserpine-treated tissues. The concentrations of these ions were also not significantly affected by reserpine treatment. The concentrations of Na^+ and K^+ were calculated to be 46.10 ± 2.60 ($n = 6$) and 122.03 ± 4.95 ($n = 6$) mmol kg^{-1} i.c. water, respectively, in control tissues, and 44.79 ± 2.87 ($n = 6$) and 113.97 ± 6.83 ($n = 6$) mmol kg^{-1} i.c. water, respectively in reserpine-treated tissues.

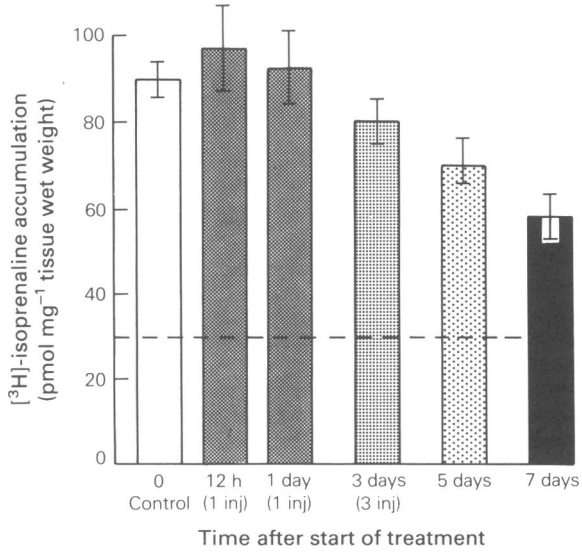

Figure 4 The time course of the change in extraneuronal accumulation of [^{3}H]-isoprenaline into atria. Each column represents the mean, and vertical lines s.e.mean, of the accumulation by 6 tissues after 90 min of incubation at 37°C. The broken line shows the level of accumulation into reserpine-treated tissues measured at 0°C (see Table 1). The part of the column in the histogram above this line therefore represents the extraneuronal uptake.

The efflux of [^{3}H]-isoprenaline after reserpine treatment

The effect of reserpine treatment on the efflux of [^{3}H]-isoprenaline from the rat atria is shown in Figure 3. The initial efflux of label from the preloaded tissues was very rapid. In fact this initial portion represented 40–50% of the total radioactivity released from the tissue over the 30 min period. The [^{3}H]-isoprenaline was released in two phases with half-times of 3.0 and 16.0 min. At no time throughout the period measured was there a significant difference between the release from control tissues and that from reserpine-treated tissues.

Time course for the loss of extraneuronal uptake after reserpine treatment.

The time course for the change in uptake into atria was examined after treatment with reserpine for various times up to 7 days (see Figure 4). It is clear that the effects of reserpine on the extraneuronal uptake were not immediate, but took days to develop. In fact the uptake into tissues from treated animals only became significantly different from the control after 3 days ($P < 0.05$). The difference continued to increase over successive days ($P < 0.01$). The study was not continued for more than 7 days because of the declining health of the animals.

Discussion

Initial studies using the vas deferens showed that reserpine treatment resulted in a decrease in the accumulation of [^{3}H]-isoprenaline, and it was suggested that the adrenergic nerves themselves play a role in the regulation of the extraneuronal uptake (Morton & Mills, 1981). However, the evidence for the role of adrenergic nerves in the control of the uptake mechanism is dependent upon the validity of several assumptions made about the effect of reserpine treatment on the properties of the tissue being studied. In this paper, the rat atrium has been used as a model of extraneuronal uptake, and an attempt has been made to clarify the nature of the reserpine-induced change in the extraneuronal accumulation of [^{3}H]-isoprenaline.

Reserpine treatment was selected as a means of pharmacological denervation for several reasons. Firstly, its chronic administration causes a widespread and extensive adrenergic denervation, and although

some less pleasant side-effects accompany its chronic administration, it is easy to administer. Secondly, it provided a useful basis for comparison with the work of Geffen (personal communication), and of Salt & Iversen (1973), who used 6-hydroxydopamine as denervation tools. Thirdly, reserpine was chosen because of the interesting early observations by Kopin and his co-workers, showing that in reserpine pretreated rats a proportional increase in deaminated metabolites and a decrease in *O*-methylated metabolites occurred (Kopin *et al.*, 1962; Kopin & Gordon, 1963). This effect was attributed to increased intraneuronal metabolism by MAO, but it is equally possible that a metabolic effect mediated by a reserpine-induced change in extraneuronal uptake may have contributed to these observations.

Unfortunately, reserpine treatment is not an ideal means of sympathetic denervation. Reserpine treatment has been shown to have deleterious effects on the neuronal membrane and possibly on intraneuronal mitochondria (Zaimis, 1961; Wilkens *et al.*, 1967), although these effects vary widely and depend on the species, dose and duration of treatment used. It has also been suggested that reserpine treatment may affect the non-neuronal effector tissues (Carrier *et al.*, 1967, Iwayama *et al.*, 1973). In view of this, a number of possible factors which might have resulted in a change in the measured accumulation of [^{3}H]-isoprenaline were investigated.

Since the extraneuronal uptake mechanism of rat heart shows a pronounced sensitivity to steroids (Iversen & Salt, 1970), a reduction in corticosterone sensitivity would support a suggestion that the extraneuronal mechanism had indeed been affected. Tissues from control and reserpine-treated rats (7 days) were equilibrated and then incubated in media which contained 100 μM corticosterone. This concentration of corticosterone was chosen because it causes a near maximal inhibition of extraneuronal uptake into rat heart (Iversen & Salt, 1970; Uhlig *et al.*, 1976). These experiments showed that the corticosterone-sensitive uptake mechanism did not apper to contribute to the accumulation measured at 0°C, and that at 37°C a large proportion of the extraneuronal uptake is inhibited by *in vitro* corticosterone. However, a significant component of extraneuronal uptake remained which appeared to be resistant to corticosterone inhibition. There was a significant difference between the accumulation measured in the presence of corticosterone at 37°C and that at 0°C. In the presence of corticosterone the extraneuronal uptake was inhibited by 67%. Both reserpine treatment and corticosterone treatment resulted in the loss of about half of the extraneuronal uptake. Incubating the reserpine-treated tissues in corticosterone did not result in a further decrease in the accumulation of [^{3}H]-isoprenaline. Thus it appears to be the corticosterone-sensitive component of extraneuronal uptake that was lost after chronic reserpine treatment.

Although it is generally accepted that the affinity of the neuronal uptake mechanism for isoprenaline is very low, and that isoprenaline appears to be specifically accumulated by extraneuronal tissues (Callingham & Burgen, 1966; Trendelenburg, 1976), some results have shown that cocaine can significantly reduce accumulation of isoprenaline in the saphenous vein, ear artery and aorta (Branco & Osswald, 1980; Head *et al.*, 1980; Branco *et al.*, 1981), thus suggesting that [^{3}H]-isoprenaline may also be taken up into the sympathetic nerves. The presence of cocaine at a concentration known to block neuronal uptake of adrenaline and noradrenaline (100 μM) had no effect on the accumulation of isoprenaline into atria. These results support the assumption that isoprenaline is not a substrate for a cocaine-sensitive accumulating mechanism, and show that the decrease in [^{3}H]-isoprenaline accumulation is not due to the loss of uptake into the neurone. They support the use of isoprenaline as a model catecholamine for extraneuronal uptake.

While the corticosterone-sensitivity of the component of accumulation lost after reserpine treatment is strongly supportive of the suggestion that reserpine treatment results in a decrease in extraneuronal uptake, it must be recognized that the change induced by reserpine treatment may also be explained by a change in other compartments accessible to isoprenaline and/or a change in the rate of efflux of the [^{3}H]-isoprenaline from the tissues. In fact the suggestion that the change in accumulation seen after reserpine treatment is due to a decrease in the extraneuronal uptake is dependent upon the validity of several assumptions made about the effect of reserpine treatment on the properties of the tissues themselves. These assumptions are that reserpine did not (a) have a direct effect on the extraneuronal uptake or storage; (b) alter the volume of the extracellular space at 37°C; (c) alter extraneuronal uptake indirectly, as a result of tissue atrophy or electrolyte imbalance; or (d) increase the efflux of isoprenaline from the tissues. The validity of these assumptions was examined.

As there was no apparent dehydration of the tissues after reserpine treatment, it was concluded that most of the loss of weight of the animal was due to a combination of the anorexia and the increased catabolism induced by the reserpine treatment. There was no change in the accumulation of [^{3}H]-isoprenaline into the tissues from fasted rats when compared with that in control rats. As the extraneuronal uptake was calculated per mg tissue (wet weight) these results showed that the decrease in extraneuronal uptake seen after reserpine treatment was not a direct effect of the loss of body weight or the weight loss of the tissues after reserpine treatment. Thus although the weight

loss during reserpine treatment was considerable, this was not a primary cause of the observed changes in extraneuronal uptake.

The methods used in the uptake studies relied on the fact that the extraneuronal uptake is temperature dependent (Gillespie *et al.*, 1970; Bönisch *et al.*, 1979; Janssens *et al.*, 1981). However, a lowering of the temperature may also lead to a reduction of accumulation independent of its effect on the extraneuronal uptake mechanism. My preliminary studies included a comparison of the extracellular space measurable at 37°C and 0°C (unpublished data). No effect of temperature on the [^{14}C]-inulin space was found. Despite this, it was recognized that even a slight change in extracellular space would alter the calculation of extraneuronal uptake. In view of the effects of reserpine on electrogenic and ionic properties of cells (Taylor *et al.*, 1976; Abel *et al.*, 1981), and the relationship between the extracellular space and cell volume (Macknight & Leaf, 1978), direct measurements of the effect of reserpine treatment on the extracellular space were made. However, no significant difference between the extracellular space in tissues from control and reserpine-treated rats was found. Thus the change in extraneuronal uptake following reserpine treatment was not due to a reserpine -induced change in the extracellular space.

Although the underlying mechanism for extraneuronal uptake is not well understood, there is evidence for the involvement of Na^+ and K^+ ions (see Gillespie, 1976; Trendelenburg, 1980). It has been suggested that reserpine treatment alters the permeability of cell membranes to Na^+ and K^+; thus the balance of ions and water in tissues from reserpine-treated rats may be disturbed (Martinez *et al.*, 1979a,b; Stewart & Jobe, 1981). Since extraneuronal uptake is at least partially dependent on the resting membrane potential (Major *et al.*, 1978) it is possible that the changes in extraneuronal uptake are due to reserpine-induced ionic changes. Unfortunately it is very difficult to measure the concentration of intracellular ions, and this has not been attempted. However, it was considered worthwhile to measure the effect of reserpine treatment on the electrolyte content of the tissues. No changes in either the water or electrolyte composition or on the ionic concentrations in the atria were detected. Thus it is unlikely that the change in extraneuronal uptake induced by reserpine treatment was due to a marked disturbance of the electrolyte balance in tissues from treated animals.

These results show that the change in accumulation of [^{3}H]-isoprenaline which follows reserpine treatment is due to a time-dependent loss of a corticosterone-sensitive component of the extraneuronal uptake mechanism. This change is not due to a direct effect of the drug on the effector cell or the extraneuronal uptake mechanism.

These experiments support the suggestion that the extraneuronal uptake is dependent on a functional adrenergic innervation. However, they do not allow any firm conclusion to be drawn about the factors that induce these changes, since, although the effect of reserpine treatment is confined to the depletion of endogenous catecholamines, the neurones themselves are not destroyed, and the synthetic pathways remain intact. The change in extraneuronal uptake may be due to the loss of any one of a number of factors, such as the influence of the catecholamines themselves, the activity of the nerve, or a trophic factor secreted from the neurone. Also, reserpine depletes central catecholamines and thus affects central mechanisms which have peripheral effects, such as temperature regulation, blood pressure regulation and some hormonal secretions.

While the cause of the change in extraneuronal uptake has not been shown here, these investigations show clearly that extraneuronal uptake is not fixed at a single functional level. This is particularly interesting, because although the contention that extraneuronal uptake has a physiological role has become more popular in recent years, its physiological significance is still a matter for considerable debate. The fact that the level of the uptake can vary lends support to the suggestion that the uptake may play a significant role in the inactivation of peripheral catecholamines.

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(Received March 26, 1985.
Revised May 9, 1985.
Accepted May 15, 1985.)