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THE EXTRANEURONAL ACCUMULATION OF ISOPRENALINE IN TRACHEA AND ATRIA OF GUINEA-PIG AND CAT: A FLUORESCENCE HISTOCHEMICAL STUDY

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1 The Falck-Hillarp histochemical technique was used to locate extraneuronal sites of accumulation of isoprenaline in trachea and atria from guinea-pig and cat. With a tissue exposure time to formaldehyde gas of 3 h, isoprenaline was located as green fluorescence.

2 Quantitative microphotometry was used to measure fluorescence intensity within cells in the trachealis smooth muscle and the atrial myocardium of both species.

3 After incubation of tissues in 50 μM isoprenaline, specific fluorescence was seen in trachealis smooth muscle of both species and in the atrial myocardium of cat but not guinea-pig. In both species, fluorescence was also seen in the chondroblasts of the tracheal cartilage and in blood vessels in all tissues.

4 In trachealis smooth muscle of both species and in cat atrial myocardium, fluorescence brightness, resulting from incubation of tissues in 50 μM isoprenaline was significantly increased by 200 μM β-thujaplicin, an inhibitor of catechol-*O*-methyl transferase (COMT). In the presence of β-thujaplicin, fluorescence was not visible in guinea-pig atrial myocardium with 50 μM isoprenaline, although fluorescence brightness measured in myocardial cells was now greater than that in corresponding controls.

5 The fluorescence intensity seen in cat and guinea-pig trachealis smooth muscle cells and in cat atrial myocardial cells after incubation in 50 μM isoprenaline was decreased significantly in the presence of phenoxybenzamine (100 μM). In guinea-pig atria, phenoxybenzamine had no effect on myocardial fluorescence. Fluorescence intensity was also decreased if the incubation with isoprenaline was carried out at 0°C or if the post-incubation washing temperature was 37°C instead of 0 to 2°C.

6 The results demonstrate that the fluorescence histochemical technique can be used to locate isoprenaline in tissues. They also indicate that guinea-pig and cat trachealis smooth muscle cells and cat atrial myocardial cells can accumulate isoprenaline (a) by a mechanism sensitive to phenoxybenzamine and (b) into sites in which COMT plays a functional role in inactivating isoprenaline at the concentration used in these histochemical experiments (50 μM). In contrast, the guinea-pig atrial myocardial cells may have a minimal capacity to accumulate isoprenaline by a phenoxybenzamine-sensitive uptake mechanism.

Introduction

In studies on the extraneuronal uptake of catecholamines, it is preferable to use isoprenaline rather than noradrenaline as substrate, since isoprenaline has a higher affinity for this uptake mechanism and is not a substrate for either neuronal uptake or monoamine oxidase (Gillespie, 1976; Trendelenburg, 1976). In fluorescence histochemical studies, the extraneuronal accumulation of noradrenaline has been examined in a number of tissues (Avakian & Gillespie, 1968; Farnebo & Malmfors, 1969; Clarke, Jones & Linley, 1969; Gillespie, Hamilton & Hosie, 1970; Burnstock, McLean & Wright, 1971; Jacobowitz & Brus, 1971; O'Donnell & Saar, 1973; Gillespie & Towart, 1973), but there are no previous reports of this technique having been used to study directly the extraneuronal accumulation of isoprenaline. In the present study, the specific fluorescence resulting from isoprenaline accumulation was demonstrated in tissues following

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exposure to gaseous formaldehyde according to the Falck-Hillarp technique (Falck, 1962). The technique, combined with quantitative microphotometry, has been used to compare the localization of isoprenaline in trachea and atria from two species, cat and guinea-pig, and to study some of the properties of this accumulation.

A preliminary account of this work was presented to the Australasian Society for Clinical and Experimental Pharmacologists (Anning, Bryan & O'Donnell, 1978).

Methods

Adult, female guinea-pigs (300 to 450 g) and cats (2 to 4 kg) were pretreated with reserpine (1.6 μmol/kg, i.p.) 24 h before the experiment to deplete endogenous catecholamines. Guinea-pigs were killed by a blow on the head and the trachea and heart removed. Cats were anaesthetized with ether, killed by opening the chest and rupturing the diaphragm, and the trachea and heart then removed.

Guinea-pig and cat tracheae were cut into 5 to 7 intact rings. Because of the size of cat tracheae, the rings were trimmed of much of the cartilage, but an intact ring was maintained to facilitate histological sectioning. Right atria of cats and both atria from guinea-pigs were used. Atria were cut into thin segments.

All tissues were initially washed in two changes of 10 ml Krebs solution aerated with 95% O_2 and 5% CO_2 over a period of 30 min to remove blood and mucus from the tissues. They were then incubated for 30 min in 10 ml of aerated Krebs solution only (controls) or containing the appropriate concentration of isoprenaline. The incubation temperature was 37°C unless otherwise indicated and this Krebs solution contained an additional 0.57 mM ascorbic acid. Tissues were then washed in 3 changes of 10 ml of ice-cold (0 to 2°C) aerated Krebs solution for a total time of 30 minutes. The effect of carrying out this washing procedure at 37°C was also examined. In experiments where inhibitor drugs were used (phenoxybenzamine and/or β-thujaplicin) these were included in the Krebs solution throughout the experimental procedure (pre-incubation washing, incubation with isoprenaline and wash-out).

Tissue sections were then prepared for fluorescence histochemistry by the Falck-Hillarp technique (Falck, 1962) as described by O'Donnell & Saar (1973) except that the exposure time to formaldehyde gas was 3 h at 80°C. Tissues, embedded in Bioloid Tissue Embedding Medium (m.p. 56 to 58°C VWR Scientific, U.S.A.), were cut into 7 μm sections and mounted in warm liquid paraffin on warm microscope slides. Sections were viewed for fluorescence by Ploem incident light illumination. The exciting light, which was the 405 nm line of a HBO 100 W mercury vapour lamp, was isolated by a Leitz 3 mm BG12 excitation filter and BG38 heat absorbing and red suppression filter. The light passed through the vertical illuminator which contained a dichroic beam-splitting mirror TK455 and built-in suppression filter K460. A K490 suppression filter was placed in the filter slide. Photographs were taken with a × 25 water immersion objective on Kodak Tri-X Pan film at constant exposure time within each experiment and all prints from one experiment were printed under the same conditions, so that a visual comparison of brightness could be obtained.

In experiments in which fluorescence brightness was quantified, areas 1.2 μm square were measured in trachealis smooth muscle or atrial myocardial cells by use of a × 100 oil immersion objective, × 12.5 eyepieces and the variable diaphragm present in the Leitz MPV microphotometer. The output from the MPV was displayed on a digital voltmeter linked to an automatic printer. The fluorescence standard used was a slice of uranium glass mounted on a microscope slide with a grid on the reverse side. A known point on this standard was measured frequently during the experiments to correct for variations in excitation intensity due to fluctuations in the mercury lamp. Corrections were made with the fine adjustment on the high voltage power supply to the photomultiplier so that a predetermined standard voltmeter reading was obtained. All measurements on tissue sections were made on coded slides, the experimental treatments being revealed only when all measurements were completed. Duplicate measurements on a minimum of four different tissue sections were made for each treatment. Care was taken not to measure any part of a section that had previously been exposed to the exciting light. Means ± s.e. means of fluorescence brightness readings from tissues from a number of animals were calculated.

The sodium borohydride reduction test for the specificity of catecholamine fluorescence was carried out on guinea-pig tracheal rings incubated with 50 μM or 500 μM isoprenaline. The method was based on that of Corrodi, Hillarp & Jonsson (1964) except that wax was melted off the sections rather than removed in xylene since xylene removed isoprenaline fluorescence.

In model experiments, dried layers of bovine serum albumin containing no amine (controls), noradrenaline, adrenaline, isoprenaline or methoxyisoprenaline were prepared on microscope slides according to the method of Jonsson (1967). The slides were exposed to formaldehyde gas at 80°C for varying times. Liquid paraffin was used as the mounting medium and fluorescence measured as described for tissue experiments.

Means ± s.e. means of fluorescence brightness readings were calculated.

The following drugs were used: (−)-adrenaline bitartrate (Fluka); bovine serum albumin (Commonwealth Serum Laboratories); (±)-isoprenaline sulphate (Sigma); (±)-methoxyisoprenaline hydrochloride (Boehringer-Ingelheim); (−)-noradrenaline bitartrate (Sigma); phenoxybenzamine hydrochloride (Smith, Kline & French); reserpine (ampoules of Serpasil, Ciba); β-thujaplicin (Koch-Light). Stock solutions (10 mM) were made on the day of the experiment. Phenoxybenzamine was dissolved in absolute ethanol containing 0.001 ml 10 M HCl per ml to give a 100 mM stock solution. β-Thujaplicin was dissolved in a small quantity of warm propylene glycol before dilution. Dilutions were made in Krebs solution of the following composition (mM): NaCl 114, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25, glucose 11.7 and ascorbic acid 1.14.

Statistical analyses

All comparisons were made by the paired *t* test unless indicated otherwise in the text or Tables.

Results

Specificity of the fluorescence

Formaldehyde-induced green fluorescence was seen in model experiments with noradrenaline, adrenaline and isoprenaline but not methoxyisoprenaline (Table 1A). Fluorescence was also seen at various sites in tissues which had been incubated in isoprenaline (see below), but not in control tissues (no isoprenaline). Fluorescence was not seen in tissues that had been incubated in isoprenaline but not exposed to formaldehyde. Fluorescence was lost if tissue sections were treated with water but it could be regenerated on heating the slide. Fluorescence was resistant to 90% isopropyl alcohol but was removed when the sections were treated for 5 min with 0.1% sodium borohydride in 90% isopropyl alcohol, thoroughly washed with solvent and dehydrated (50°C, 30 min). Re-exposure of tissue sections to formaldehyde gas at 80°C for 3 h regenerated the fluorescence.

Exposure time to formaldehyde gas

In model experiments, there was a significant increase in fluorescence brightness in isoprenaline spots compared with the corresponding controls (2% bovine serum albumin only) after 1, 3 and 5 h exposure to formaldehyde gas (Table 1A). The fluorescence intensity in isoprenaline spots was significantly greater if the exposure time was 3 h, as is used for adrenaline, rather than 1 h, as used for noradrenaline ($t = 3.54$, d.f. 62, $P < 0.001$, Student's *t* test). If the exposure time was extended to 5 h, there was no further increase in fluorescence brightness. This was confirmed in a subsequent experiment in which isoprenaline fluorescence in guinea-pig trachealis smooth muscle was measured after 1, 3 and 5 h exposure to formaldehyde

Table 1 Fluorescence brightness measurements (A) in model experiments, (B) in tissue experiments.

	Fluorescence brightness (arbitrary units) Specified exposure time to formaldehyde		
	1 h	3 h	5 h
A†			
Controls (no amine)	43 ± 2.0	57 ± 4.5	54 ± 3.6
Noradrenaline	1014 ± 36.1***	—	—
Adrenaline	—	569 ± 38.8***	—
Isoprenaline	205 ± 10.9***	406 ± 51.4***	395 ± 25.0***
Methoxyisoprenaline	—	49 ± 2.2	—
B‡			
Controls (no amine)	39 ± 2.9	51 ± 2.4	51 ± 1.3
Isoprenaline	124 ± 12.2**	340 ± 11.1***	297 ± 16.4***

† Fluorescence brightness measured in 1 µl spots of 50 µM noradrenaline, adrenaline, isoprenaline or methoxyisoprenaline in 2% bovine serum albumin or in 1 µl spots of bovine serum albumin only (controls). Each value represents mean ± s.e. mean for duplicate measurements from 16 spots, i.e. 32 measurements from different locations.

‡ Fluorescence brightness measured in trachealis smooth muscle of tracheal segments taken from guinea-pigs which were pretreated with 1.6 µmol/kg reserpine. Segments were incubated in Krebs solution (controls) or in Krebs containing 50 µM isoprenaline for 30 min followed by washing in Krebs solution at 0°C for 30 minutes. Values represent mean ± s.e. mean of measurements from tissues taken from 4 guinea-pigs.

Fluorescence brightness measurements which are significantly greater than the controls at same exposure time are indicated by asterisks (A Student's *t* test; B paired *t* test): ** $0.01 > P > 0.001$; *** $P < 0.001$.

(Table 1B). Therefore, 3 h exposure to formaldehyde gas was used throughout the remainder of the study.

Localization of isoprenaline fluorescence

In sections from guinea-pig and cat tracheal segments incubated in 50 μM isoprenaline but without a catechol-*O*-methyl transferase (COMT) inhibitor present, specific fluorescence (not visible in the controls with no isoprenaline) was seen in trachealis smooth muscle, in blood vessels and in the chondroblasts of the cartilage (Figure 1). In cat atrial sections under similar conditions, bright fluorescence was seen in the atrial myocardium and in the smooth muscle of blood vessels (Figure 1). In contrast, fluorescence was not seen in myocardial cells of guinea-pig atria even

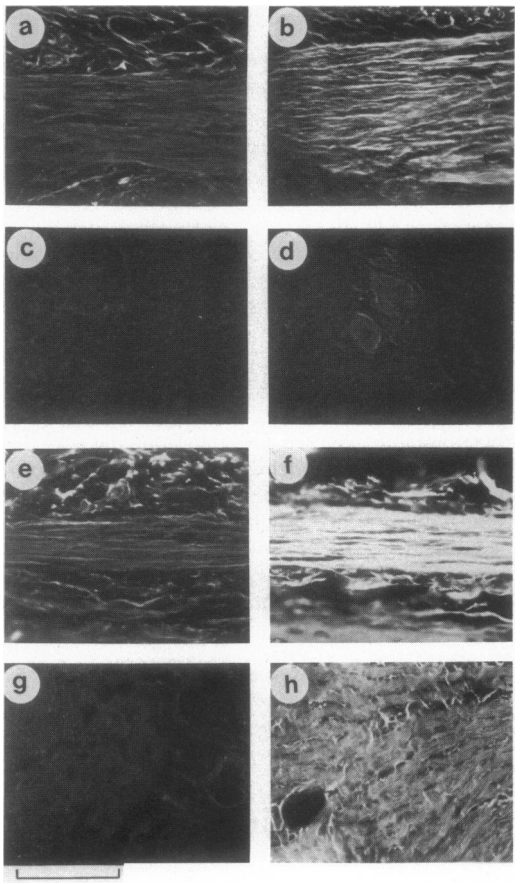

Figure 1 Fluorescence photomicrographs from a typical experiment to illustrate the increase in fluorescence brightness in guinea-pig and cat trachealis smooth muscle and in cat, but not guinea-pig, atrial myocardium after incubation of tissues in isoprenaline. Photomicrographs (a) and (b) are of guinea-pig trachealis smooth muscle, (c) and (d) of guinea-pig atria, (e) and (f) of cat trachealis smooth muscle and (g) and (h) of cat atria. The lefthand photomicrographs represent sections taken from tissue segments incubated in Krebs solution (without isoprenaline) and the righthand ones represent sections taken from different segments of the same tissues incubated in 50 μM isoprenaline. The guinea-pig and cat received 1.6 μmol/kg reserpine 24 h previously so that no adrenergic nerves are present. Calibration bar = 100 μm.

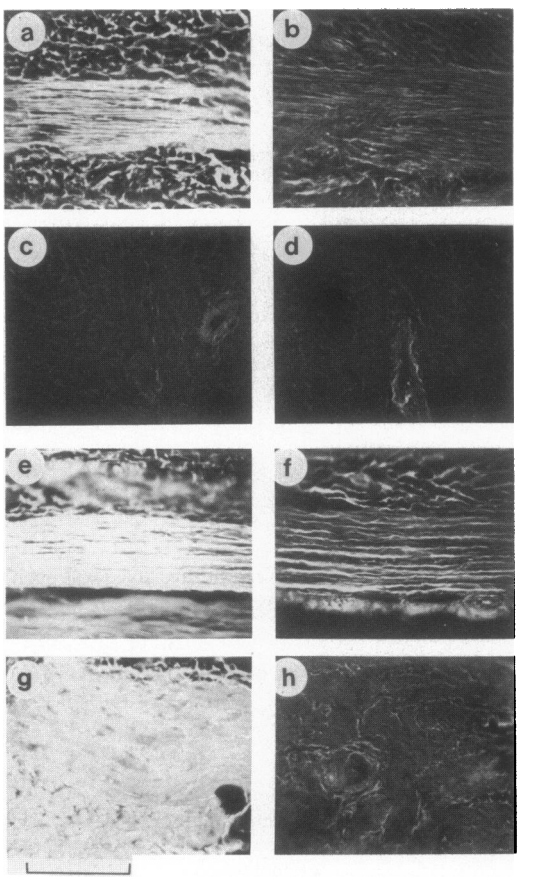

Figure 2 Fluorescence photomicrographs of guinea-pig trachealis smooth muscle (a) and (b), guinea-pig atria (c) and (d), cat trachealis smooth muscle (e) and (f) and cat atria (g) and (h). The photomicrographs represent sections taken from further tissue segments from the same animals and the same experiment as is shown in Figure 1. The Krebs solution contained 200 μM β-thujaplicin for these segments. The lefthand photomicrographs are for incubation in 50 μM isoprenaline and the righthand ones are for incubation with 50 μM isoprenaline in the presence of 100 μM phenoxybenzamine. The reduction by phenoxybenzamine of fluorescence due to isoprenaline in guinea-pig and cat trachealis smooth muscle and in cat atrial myocardium is illustrated. Calibration bar = 100 μm.

though fluorescence associated with blood vessels could be seen readily (Figure 1).

Effect of β-thujaplicin on isoprenaline fluorescence

Inclusion of 200 μM β-thujaplicin in the Krebs solution had no effect on control (no isoprenaline) fluorescence readings in any tissues (Table 2). When guinea-pig and cat tracheal segments were incubated in 50 μM isoprenaline in the presence of 200 μM β-thujaplicin, there was a visual increase in fluorescence brightness (compared with tissues incubated in isoprenaline in the absence of β-thujaplicin) in trachealis smooth muscle cells (compare Figures 1b and 2a; Figures 1f and 2e). This was also reflected in the fluorescence measurements (Table 2, guinea-pig: $t = 10.81$, d.f. 3, $0.01 > P > 0.001$; cat: $t = 12.04$, d.f. 3, $0.01 > P > 0.001$). In cat atria there was visual enhancement of isoprenaline fluorescence in the myocardial cells by β-thujaplicin (compare Figures 1h and 2g), although, when measured, this failed to reach statistical significance ($t = 3.0$, d.f. 3). In guinea-pig atria, fluorescence was not visible in myocardial cells (Figure 2c) and β-thujaplicin caused no increase in fluorescence brightness in these cells (compare Figures 1d and 2c; Table 2, $t = 0.54$, d.f. 3). However, the fluorescence brightness measured in myocardial cells was now greater than that in the corresponding controls ($t = 4.21$, d.f. 3, $0.05 > P > 0.01$). Fluorescence brightness in blood vessels in atrial sections was visually enhanced by β-thujaplicin, although this was not measured. Inclusion of 200 μM β-thujaplicin in the Krebs solution enabled fluorescence to be seen in trachealis smooth muscle of both species and in myocardial cells of cat when a lower concentration, 10 μM, of isoprenaline was used. This was not possible in the absence of β-thujaplicin.

Effects of phenoxybenzamine and of treatments on isoprenaline fluorescence

Comparison of fluorescence brightness due to incubation in isoprenaline (50 μM) with and without phenoxybenzamine (100 μM) but in the presence of β-thujaplicin (200 μM) showed that phenoxybenzamine caused a significant reduction in fluorescence brightness in guinea-pig trachealis smooth muscle (compare Figures 2a and b; Table 2, $t = 28.20$, d.f. 3, $P < 0.001$), in cat trachealis smooth muscle (Figures 2e and f; Table 2, $t = 41.78$, d.f. 3, $P < 0.001$) and in cat atrial myocardial cells (Figures 2g and h; Table 2, $t = 6.54$, d.f. 3, $0.01 > P > 0.001$). Phenoxybenzamine (100 μM) did not reduce fluorescence readings back to control (no isoprenaline) values in guinea-pig or cat trachealis smooth muscle cells or in cat atrial myocardial cells (Table 2). In guinea-pig atria, phenoxybenzamine had no effect on measured myocardial fluorescence in the presence of β-thujaplicin (Figures 2c and d; Table 2, $t = 0.98$, d.f. 3).

Fluorescence brightness in blood vessels in all sections, including guinea-pig atria, appeared visually to be reduced but this was not quantified. Phenoxybenzamine (100 μM) also appeared to reduce the fluorescence brightness in the chondroblasts of tracheal cartilage in both species, although this was not quantified.

Table 2 Fluorescence brightness measured in cells in sections from guinea-pig and cat tissues

	Fluorescence brightness (arbitrary units)			
	Trachealis smooth muscle cells		*Atrial myocardial cells*	
	No β-Thuj	*β-Thuj*	*No β-Thuj*	*β-Thuj*
Guinea-pig				
No Iso	111 ± 3.6	117 ± 1.2	126 ± 15.7	118 ± 13.8
Iso	463 ± 29.9***	774 ± 15.2***	135 ± 15.1	139 ± 10.4*
Iso + Pbz	—	295 ± 10.6***	—	132 ± 15.1*
Cat				
No Iso	169 ± 3.6	166 ± 4.7	143 ± 10.3	141 ± 10.7
Iso	691 ± 23.3***	992 ± 8.4***	312 ± 38.8**	442 ± 21.9**
Iso + Pbz	—	392 ± 7.4***	—	227 ± 16.1**

Results represent mean ± s.e. mean of readings from tissues taken from 4 guinea-pigs and 4 cats. Tissues were incubated in 50 μM isoprenaline (Iso) as indicated. The concentration of β-thujaplicin (β-Thuj) in the Krebs solution was 200 μM and of phenoxybenzamine (Pbz) 100 μM. All animals received 1.6 μmol/kg reserpine 24 h before the experiment.

Fluorescence brightness measurements which are significantly greater than the corresponding controls (No Iso) are indicated by asterisks (paired t test): * $0.05 > P > 0.01$; ** $0.01 > P > 0.001$; *** $P < 0.001$.

In experiments in which incubation with isoprenaline (50 μM) was carried out at 0 to 2°C, fluorescence brightness at all sites in all tissues was less than at 37°C. Fluorescence was much reduced also if the post-incubation washing treatment was carried out at 37°C instead of at 0 to 2°C.

Discussion

This study has demonstrated that the Falck-Hillarp fluorescence histochemical technique can be used to examine the extraneuronal accumulation of isoprenaline in tissues. Fluorescence was initially observed in protein model experiments with isoprenaline and then in tissues incubated in isoprenaline. The optimum exposure time to formaldehyde gas was established as 3 hours. The fluorescence observed in tissues was specific in that it was absent from control tissues and it also showed characteristics (outlined in the Results section) of fluorescence of fluorophores derived from catecholamines (Corrodi *et al.*, 1964; Corrodi & Jonsson, 1967).

The technique was used to compare the extraneuronal accumulation of isoprenaline in two tissues (trachea and atria) of two species (guinea-pig and cat). After incubation of tissue segments with 50 μM isoprenaline, green fluorescence (not present in controls) was seen in the smooth muscle of trachea from both species and in the atrial myocardium of cats. Fluorescence brightness measured in cells at these sites was significantly less if tissues were incubated with isoprenaline in the presence of phenoxybenzamine (100 μM), an inhibitor of extraneuronal uptake. Fluorescence brightness was visually much less at these sites if incubation with isoprenaline was carried out at 0 to 2°C instead of 37°C and also if the post-incubation washing of the tissues with Krebs solution was carried out at 37°C instead of at 0 to 2°C. The properties of the accumulation resembled those reported previously for the extraneuronal accumulation of noradrenaline including sensitivity to temperature and inhibition by phenoxybenzamine (Gillespie, 1976). That the fluorescence was due to isoprenaline and not its *O*-methylated metabolite, was confirmed by the observation that in model experiments methoxyisoprenaline did not fluoresce. Fluorescence brightness was enhanced significantly in the presence of a COMT inhibitor, 200 μM β-thujaplicin, indicating an increased accumulation of unchanged isoprenaline. Thus, in trachealis smooth muscle cells of guinea-pig and cat and in atrial myocardial cells of cat, isoprenaline appeared to accumulate in sites containing COMT and the enzyme played a functional role in the inactivation of isoprenaline at the concentration used (50 μM).

In contrast, no fluorescence was seen in the myocardium of guinea-pig atria after incubation in the same concentration of isoprenaline (50 μM) whether or not β-thujaplicin was present. In the presence of β-thujaplicin, measurements of brightness suggested that an increase in fluorescence due to isoprenaline when compared with no isoprenaline controls had occurred but this fluorescence was not decreased by phenoxybenzamine. In blood vessels in the atrial sections, on the other hand, the bright fluorescence was enhanced visually by β-thujaplicin and reduced by phenoxybenzamine. Thus the slight fluorescence measured in the guinea-pig atrial myocardial cells differed distinctly from that measured in trachealis smooth muscle and cat atrial myocardium and observed in blood vessels. This might suggest that the fluorescence seen in guinea-pig atrial myocardium was due to a non-specific binding of isoprenaline rather than to an intracellular accumulation by an uptake mechanism. Further experiments, particularly with higher concentrations of isoprenaline and with various inhibitor drugs, are required to determine this. Nevertheless, these fluorescence results clearly indicate that the atrial myocardial cells of guinea-pig have a much lower capacity to accumulate isoprenaline intracellularly than the others studied. At present we can only speculate on the reason why a phenoxybenzamine-sensitive fluorescence was not seen in myocardial cells in guinea-pig atria. The myocardial cells in the guinea-pig may lack an extraneuronal transport system to transfer isoprenaline into the cells. Alternatively, isoprenaline may enter the cells and either remain unbound (not studied) or the rate of its metabolism by intracellular COMT may exceed the capabilities of the uptake mechanism so that there may be insufficient accumulation of unchanged isoprenaline for observation by the fluorescence technique. However, the fact that little fluorescence was seen in guinea-pig myocardial cells when COMT was inhibited suggests that COMT metabolism is not the factor limiting the accumulation of isoprenaline in these cells. From different types of experiments, Bonisch & Trendelenburg (1974) and Trendelenburg (1976) reported that the perfused heart of guinea-pig, unlike that of rat and cat, had a limited capacity to accumulate [^{3}H]-isoprenaline and that this was not increased by inhibition of COMT. Jarrott (1970) noted considerable *O*-methylation of 3,4-dihydroxybenzoic acid by homogenates of guinea-pig heart and so provided direct evidence for the presence of COMT activity in this tissue. However, significant *O*-methylation of noradrenaline when perfused through the guinea-pig heart could not be demonstrated. Subsequently, Jarrott (1973) suggested that the guinea-pig heart lacked the extraneuronal transport system necessary for exposure of noradrenaline to COMT. The inability to demonstrate a phenoxybenzamine-sensitive extraneuronal accumulation of

isoprenaline in guinea-pig myocardial cells in the present fluorescence histochemical study is compatible with the findings of these authors. The variations in the extraneuronal accumulation of isoprenaline observed between species and between tissues are in accordance with previous studies on the extraneuronal accumulation of noradrenaline which showed considerable tissue and species variability (Gillespie & Muir, 1970).

The concentration of isoprenaline used to demonstrate the extraneuronal accumulation of isoprenaline in this fluorescence study, *viz.* 50 μM, is higher than those used in pharmacological studies on the same tissues. Therefore, it cannot be presupposed that the accumulations described in this paper are those involved in modulating the concentration of isoprenaline in the region of the β-adrenoceptors in studies with isolated tissue preparations. However, it is interesting to note that the fluorescence findings are compatible with reported effects of extraneuronal uptake inhibitor drugs on pharmacological responses to isoprenaline. For example, relaxation of guinea-pig trachea and the positive inotropic and chronotropic effects of isoprenaline on cat atria (tissues shown to have a phenoxybenzamine-sensitive accumulation process) were potentiated by drugs inhibiting extraneuronal uptake (Kaumann, 1972; O'Donnell & Wanstall, 1976; Goldie, 1976), whereas the positive inotropic and chronotropic responses to isoprenaline on guinea-pig atria (a tissue shown to have limited capacity to accumulate isoprenaline) were not (Woppel & Trendelenburg, 1973; O'Donnell & Wanstall, 1976; Goldie, 1976).

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