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# Kinetics of extraneuronal uptake of isoprenaline in trachealis smooth muscle cells: comparison of rat and guinea-pig

LESLEY J. BRYAN, JULIANNE J. COLE, STELLA R. O'DONNELL*, *Pharmacology Section, Department of Physiology and Pharmacology, University of Queensland, Brisbane, Queensland 4067, Australia*

Bryan & O'Donnell (1980a) have used fluorescence microphotometry to determine the relative affinities of noradrenaline, adrenaline and isoprenaline for extraneuronal uptake specifically in smooth muscle cells. The cells examined were in the trachealis muscle of guinea-pig trachea. In the present study the kinetics of extraneuronal uptake of isoprenaline in the same cell type but in a different species, rat, has been examined.

Adult, male rats (325–425 g), pretreated with 50 mg $kg^{-1}$ 6-hydroxydopamine intravenously 24 h before the experiment, were killed by a blow on the head. The trachea was removed and cut into six to eight rings which were randomly assigned to the treatments in each experiment. The tracheal rings were washed and incubated in (±)-isoprenaline at 37 °C as described by Bryan & O'Donnell (1980a). Catechol-*O*-methyl transferase was inhibited by inclusion of 100 $\mu$M U-0521 in the Krebs solution (Bryan & O'Donnell 1979). After washing at 0–1 °C, the tissues were prepared for fluorescence histochemistry by the Falck-Hillarp technique (Falck 1962) as described by Anning et al (1979). Fluorescence intensities (in arbitrary fluorescence units, F) in areas of trachealis smooth muscle 2·5 $\mu$m square were measured and the intensity values were corrected for background fluorescence as described by Bryan & O'Donnell (1980b). All drugs and solutions were prepared as described by Bryan & O'Donnell (1980a).

In a preliminary experiment with 800 $\mu$M isoprenaline, uptake of isoprenaline by the trachealis smooth muscle cells was measured at incubation times of 2, 5, 10, 15, 20, 30 and 60 min. Uptake occurred at constant rate up to 5 min, then gradually decreased in rate at the longer incubation times. Hence, an incubation time of 4 min in isoprenaline was used in the subsequent initial rate study.

Initial rates of uptake of isoprenaline into trachealis smooth muscle cells were determined for amine concentrations of 50, 100, 200, 400 and 800 $\mu$M. Plots of mean initial rate of uptake (v) from 5 rats against isoprenaline concentration (s) (Fig. 1A) and of s/v against s (Fig. 1B) indicated that the uptake obeyed Michaelis-Menten saturation kinetics as was found for the same cells in guinea-pig (Bryan & O'Donnell 1980a). For each animal, $K_m$ and $V_{max}$ values were determined by weighted regression analysis according to the method of Wilkinson (1961). For rat trachealis smooth muscle, $K_m = 484 \pm 32{\cdot}8$ $\mu$M and $V_{max} = 441 \pm 16{\cdot}4$ F $min^{-1}$ (mean ± s.e. from 5 rats), compared with $K_m = 273 \pm 12{\cdot}1$ $\mu$M and $V_{max} = 257 \pm 6{\cdot}6$ F $min^{-1}$ in guinea-pig (Bryan & O'Donnell 1980a). The $K_m$ and $V_{max}$ values were both significantly greater in rat than in guinea-pig ($K_m$: $t = 6{\cdot}04$, d.f. 8, $P < 0{\cdot}001$; $V_{max}$: $t = 10{\cdot}41$, d.f. 8, $P < 0{\cdot}001$; Student's *t*-test).

These results indicate that the extraneuronal uptake mechanism for isoprenaline in trachealis smooth muscle cells in rat has a slightly lower affinity but also a slightly higher capacity than was found in guinea-pig cells. Thus, for those experimentally used substrate concentrations less than 400 $\mu$M, the rates of uptake of isoprenaline in the two species did not differ significantly, but at higher concentrations the rate of uptake was significantly greater in rat than in guinea-pig. It could be predicted from the kinetic parameters that at low isoprenaline concentrations (less than 20 $\mu$M) the reverse order would apply. These observations suggest that the small differences between the kinetic parameters of extraneuronal uptake in rat and guinea-pig trachea are unlikely to be of pharmacological signifi-

* Correspondence.

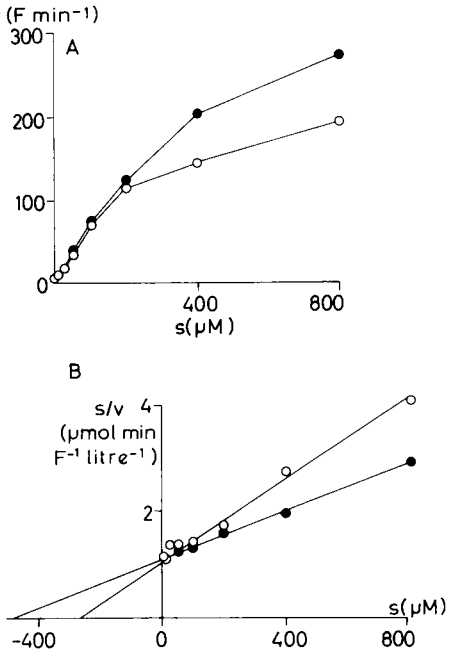


FIG. 1. Kinetic analysis of the extraneuronal uptake of isoprenaline in rat trachealis smooth muscle (●). Data obtained in the same cells in guinea-pig (○) are also shown for comparison (from Bryan & O'Donnell 1980a). (A) shows mean initial rate of isoprenaline uptake (v) in 5 rats or guinea-pigs in arbitrary fluorescence units per min (F $min^{-1}$) plotted against isoprenaline concentration (s) in $\mu$M. The standard errors of the values of v were smaller than the symbols. (B) shows values of s/v (in $\mu$mol min $F^{-1}$ $litre^{-1}$) plotted against s. The regression lines shown were obtained from the mean $K_m$ and $V_{max}$ values calculated by application of the method of Wilkinson (1961) to the results for each animal.

cance. It is known that in guinea-pig trachea inhibitors of this uptake mechanism potentiate responses to isoprenaline from which it is deduced that extraneuronal uptake can influence $\beta$-adrenoceptor biophase concentrations of isoprenaline (O'Donnell & Wanstall 1976). The authors are not aware of any results of similar experiments carried out on rat trachea.

Kinetic studies on extraneuronal uptake have been carried out by several workers in other tissues of the rat, but they used radiolabelled isoprenaline. The $K_m$ values obtained (viz. 110 $\mu$M in rat perfused heart, Bönisch 1978; 311 $\mu$M in rat submaxillary gland, Major et al 1978) are not directly comparable with the value determined in the present study, because the apparent $K_m$ values from experiments with radiolabelled amine do not relate to a particular cell type in the tissue. Since the fluorescence histochemical experiments on rat and guinea-pig described in the present study were carried out under identical conditions, it is possible that the difference found between the affinities of isoprenaline in the two species, although not great, might reflect a species difference in the carrier systems involved in catecholamine transport in the trachealis smooth muscle cells. However, further data on the affinities of catecholamines for extraneuronal uptake into specific cells of diverse organs from various species are needed before deductions can be made as to whether the uptake of catecholamines into non-neuronal cells occurs via an identical carrier system in different species and tissues.

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