Extraneuronal accumulation of isoprenaline in guinea-pig trachea, atria and uterus: a histochemical and pharmacological study

ELIZABETH N. ANNING,
STELLA R. O'DONNELL &
JANET C. WANSTALL
(introduced by I.E. HUGHES)

Pharmacology Unit, Department of Physiology, University of Queensland, Brisbane 4067, Australia

In a previous study the pharmacological responses to isoprenaline on guinea-pig isolated tracheal chain preparations were potentiated by extraneuronal uptake (ENU) inhibitor drugs (O'Donnell & Wanstall, 1976). In the present study concentration-response lines to isoprenaline were obtained on guinea-pig isolated preparations of atria (rate) in Krebs solution at 32°C; of uterus in K^+-Krebs solution at 37°C (K^+-depolarised preparation described by O'Donnell, Persson & Wanstall, 1977); and of uterus in de Jalon's solution at 27°C (inhibition of acetylcholine contractions). Phenoxybenzamine or metanephrine (50μM), used as inhibitors of extraneuronal uptake, caused the following mean changes (± s.e. mean) in the neg. log EC_{50} values for isoprenaline: *on atria* – an increase of 0.02 ± 0.04 log units ($n = 9$) by phenoxybenzamine; *on K^+-depolarised uterus* – a decrease of 0.28 ± 0.08 log units ($n = 4$) by phenoxybenzamine and a decrease of 0.39 ± 0.10 log units ($n = 4$) by metanephrine; *on uterus (acetylcholine contractions)* – a decrease of 0.01 ± 0.13 log units ($n = 3$) by metanephrine. Thus, inhibitors of extraneuronal uptake did not potentiate responses to isoprenaline on atria or uterus.

Histochemical experiments were carried out on segments of trachea, atria and uterus from guinea-pigs pretreated with reserpine (1 mg/kg i.p., 24 h previously). Tissues were incubated with isoprenaline (50 or 500 μM) (a) in Krebs solution at 37°C with or without phenoxybenzamine (100 μM) or metanephrine (500 μM) (b) in Krebs solution at 27°C and (c) in K^+-Krebs solution at 37°C. All tissues were then washed in ice-cold Krebs solution for 30 min before preparing for fluorescence histochemistry. The exposure time to formaldehyde vapour at 80°C was 3 h and fluorescence brightness measurements were made on all sections using a Leitz MPV microphotometer. In tissues incubated in isoprenaline in Krebs solution at 37°C, distinct green fluorescence was seen extraneuronally in tracheal and uterine smooth muscle but not in the atrial myocardium. The accumulation in trachea and uterus was inhibited by the extraneuronal uptake inhibitors, phenoxybenzamine or metanephrine. It was also inhibited if the incubation with isoprenaline was in K^+-Krebs (at 37°C) or was in Kerbs or de Jalon's solution at 27°C, i.e. the conditions used in the pharmacological experiments on the uterus.

The results suggest that guinea-pig tracheal and uterine smooth muscle have a capacity to accumulate isoprenaline extraneuronally but that this capacity might be very limited in the atrial myocardium. However, extraneuronal accumulation of isoprenaline in uterine smooth muscle is much reduced under the conditions used in the pharmacological experiments (low temperature or Krebs solution in which all the sodium ions are replaced by potassium ions) whereas this is not so for trachea (Krebs solution at 37°C). This may explain (a) why responses to isoprenaline were not potentiated by extraneuronal uptake inhibitor drugs on preparations of uterus whereas they were on trachea, and b) why O'Donnell *et al.*, (1977) found that there was good agreement between relative potency values (relative to isoprenaline) for ß-adrenoceptor stimulants on uterus (without inhibiting extraneuronal uptake) and on trachea (after inhibition of extraneuronal uptake).

Financial support from the National Health & Medical Research Council of Australia is gratefully acknowledged.

References

O'DONNELL, S.R. & WANSTALL, J.C. (1976). The contribution of extraneuronal uptake to the trachea-blood vessel selectivity of β-adrenoceptor stimulants *in vitro* in guinea-pigs. *Br. J. Pharmac.*, **57**, 369–373.

O'DONNELL, S.R., PERSSON, C.G.A. & WANSTALL, J.C. (1977). A comparative *in vitro* study of ß-adrenoceptor stimulants on potassium-depolarised uterine preparations from guinea-pigs. *Proc. Austr. Physiol. Pharmacol. Soc.*, **8**, 12P.