The oil immersion technique for studying the disposition of drugs inducing relaxation; influence of U-0521 and hydrocortisone on the disposition of isoprenaline

M. Q. PAIVA†, S. GUIMARÃES, *Department of Pharmacology, Faculty of Medicine, 4200 Porto, Portugal*

In 1968 Kalsner & Nickerson described the oil immersion technique for studying the disposition of adrenergic agonists. After a steady-state contraction had been produced by an agonist in aqueous solution, the bathing fluid was replaced by mineral oil to prevent loss of the agonist into the surrounding medium, and the rate of relaxation was measured. This method has been used only for studying the disposition of agonists inducing contraction. We have adopted it for studying the disposition of isoprenaline.

Segments of both lateral saphenous veins were obtained from mongrel dogs anaesthetized with pentobarbitone 30 mg kg^{-1} and helically cut strips were prepared as described by Guimarães & Osswald (1969). These were suspended in 20 ml organ baths containing Krebs-Henseleit solution, at 37 °C and bubbled with 95% O_2 + 5% CO_2. To study the relaxant effect of isoprenaline, a previous steady-state contraction is required. Acetylcholine ($2{\cdot}2 \times 10^{-4}$ M), carbachol ($2{\cdot}2 \times 10^{-4}$ M), potassium chloride ($5{\cdot}4 \times 10^{-2}$ M) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) were tested as contraction producing agents. Of these $PGF_{2\alpha}$ was the agent of choice, because it caused contractions of similar magnitude when repeatedly used in the same concentration and because it was neither inactivated by the preparation nor diffusible into the oil. The contraction caused by $2{\cdot}8 \times 10^{-6}$ M lasted for at least 7 h.

After contractions obtained with $2{\cdot}8 \times 10^{-6}$ M $PGF_{2\alpha}$—which were about 70% of the maximal response to noradrenaline—had reached a steady state isoprenaline was added to the Krebs solution. When the ensuing relaxation reached a steady state (ca 5 min after the addition of isoprenaline), the bathing solution was replaced by warmed and oxygenated oil and the time required for 50% recovery (contraction) was measured. To study the influence of U-0521 (3′, 4′-dihydroxy-2-methyl propiophenone) and hydrocortisone on the disposition of isoprenaline ($2{\cdot}4 \times 10^{-8}$ M) the drugs were added to the bathing fluid 30 min before the second addition of $PGF_{2\alpha}$ and kept in the bath during exposure to the agonist. The time required for 50% contraction in the presence of U-0521 (or hydrocortisone) was compared with that obtained in its absence.

As shown in Table 1 the time needed by the strips after exposure to isoprenaline and immersion in oil to recover 50% (t50) was concentration-dependent which indicates that the time-course of the recovery after immersion in oil can be used as an index of drug inactivation. U-0521 and hydrocortisone prolonged the t50 for recovery after relaxations caused by $2{\cdot}4 \times 10^{-8}$ M isoprenaline in a concentration-dependent way (Table 2). These facts allow us to conclude that the oil immersion technique which is a simple and adequate method for studying the disposition of adrenergic (Kalsner & Nickerson 1968) and non-adrenergic contraction inducing drugs (Paiva & Osswald 1980) is also suitable for studying the disposition of drugs inducing relaxation.

The results also show that the prolongation of the t50 caused by $1{\cdot}1 \times 10^{-4}$ M U-0521—a concentration generally recognized as able to fully block catechol-*O*-methyl transferase (COMT) (Giles & Miller 1967)—

Table 1. Influence of the concentration on the time required by the strips to recover in oil 50% (t50) after relaxation caused by isoprenaline. t50 values marked with * are different from that corresponding to $2{\cdot}4 \times 10^{-8}$ M isoprenaline $P < 0{\cdot}05$.

Isoprenaline (M)	t50 (min)	n
$2{\cdot}4 \times 10^{-8}$	$2{\cdot}1 \pm 0{\cdot}1$	45
$4{\cdot}8 \times 10^{-8}$	$2{\cdot}6 \pm 0{\cdot}3$	5
$9{\cdot}6 \times 10^{-8}$	$3{\cdot}5 \pm 0{\cdot}6$*	5

Table 2. Influence of U-0521 and hydrocortisone on the inactivation of isoprenaline. t50 = time required by the strips to recover 50% after relaxation caused by $2{\cdot}4 \times 10^{-8}$ M isoprenaline. n = number of experiments. F = ratio t50 value obtained in the presence of the drug/control t50. The values marked with * are different from the controls ($P < 0{\cdot}05$)

		t50 (min)	n	F
Control		$2{\cdot}1 \pm 0{\cdot}2$	26	—
U-0521				
10^{-6} M ×	0·6	$2{\cdot}5 \pm 0{\cdot}4$	4	1·2
,,	1·4	$4{\cdot}4 \pm 0{\cdot}5$*	3	2·1
,,	2·8	$8{\cdot}8 \pm 1{\cdot}1$*	4	4·2
,,	5·5	$25{\cdot}8 \pm 4{\cdot}0$*	3	12·3
,,	11·1	$70{\cdot}9 \pm 10{\cdot}3$*	3	33·8
,,	27·8	>80	2	>38
,,	111·2	>80	2	>38
		t50 (min)	n	F
Control		$2{\cdot}0 \pm 0{\cdot}2$	19	—
Hydrocortisone				
10^{-6} M ×	10·3	$1{\cdot}8 \pm 0{\cdot}4$	3	0·9
,,	20·6	$2{\cdot}0 \pm 0{\cdot}3$	3	1·0
,,	41·1	$2{\cdot}2 \pm 0{\cdot}2$	4	1·1
,,	82·2	$2{\cdot}8 \pm 0{\cdot}3$*	4	1·4
,,	164·4	$3{\cdot}6 \pm 0{\cdot}5$*	5	1·8

† Correspondence.

was much larger than that caused by 1.6×10^{-4} M hydrocortisone—a concentration generally accepted as sufficient to accomplish a full block of extraneuronal uptake (Iversen & Salt 1970). This can be explained assuming that, because of its relatively high lipid solubility (Mack & Bönisch 1979), isoprenaline is able to enter the cells in part by diffusion, thus partially circumventing the block by hydrocortisone of the carrier system for the transport across the membrane. Thus, if COMT is the factor generating the concentration gradient between the biophase and the medium (Trendelenburg 1972) block of the enzyme by U-0521 will keep isoprenaline molecules for a longer time in the biophase than hydrocortisone.

This difference between the effects of U-0521 and hydrocortisone can be alternatively explained if we admit that more than one *O*-methylating system exists, only one of which is hydrocortisone-sensitive as was demonstrated for the nictitating membrane (Graefe & Trendelenburg 1974). In this case U-0521 would prolong the inactivation time more than hydrocortisone because it would block the whole *O*-methylating capacity of the tissue.

The authors wish to thank Dr G. Johnson, the Upjohn Company Kalamazoo, Mich. for his generous gift of U-0521 and Prostaglandin $F_{2\alpha}$. This study was supported in part by Instituto Nacional de Investigação Científica (FmPl) and in part by NATO grant n.1369.

April 22, 1980

REFERENCES

Giles, R. E., Miller, J. W. (1967) J. Pharmacol. Exp. Ther. 157: 55–61

Graefe, K.-H., Trendelenburg, U. (1974) Naunyn-Schmiedeberg's Arch. Pharmacol. 286: 1–48

Guimarães, S., Osswald, W. (1969) Eur. J. Pharmacol. 5: 133–140

Kalsner, S., Nickerson, M. (1968) Can. J. Physiol. Pharmacol. 46: 718–730

Iversen, L. L., Salt, P. J. (1970) Br. J. Pharmacol. 40: 528–530

Mack, F., Bönisch, H. (1979) Naunyn-Schmiedeberg's Arch. Pharmacol. 310: 1–10

Paiva, M. Q., Osswald, W. (1980) J. Pharm. Pharmacol. 32: 227–228

Trendelenburg, U. (1972) in: Blaschko, H., Muscholl, E. (ed.) Catecholamines. Springer Verlag, pp. 726–761

Inhibition of antigen-induced release of prostaglandin-like material from guinea-pig trachea by antihistamines, FPL55712† and atropine

J. F. BURKA*, N. A. M. PATERSON, *Department of Medicine, Victoria Hospital, University of Western Ontario, London, Ontario, Canada, N6A 4G5*

A variety of stimuli induce the release of a prostaglandin E (PGE)-like substance from guinea-pig isolated trachea. These include histamine (Grodzinska et al 1975) and arachidonic acid (AA) as well as antigen in sensitized guinea-pigs (Burka & Paterson 1980a, b). Dexamethasone and mepacrine, both inhibitors of phospholipase A_2 (Blackwell et al 1978), inhibited the release of PGE-like material induced by histamine and antigen, but not that induced by AA, suggesting that histamine and antigen initiated prostaglandin production by providing free AA by a phospholipase A_2 mechanism (Burka & Paterson 1980b) as has been observed in guinea-pig perfused lung (Blackwell et al 1978). The present study was designed to examine by means of pharmacological antagonists whether histamine, SRS-A, or acetylcholine were responsible for the antigen-induced release of PGE-like material from guinea-pig sensitized trachea.

Male English short-hair guinea-pigs (200–250 g) were sensitized with ovalbumin 100 mg s.c. and 100 mg i.p. The trachea was removed 2–4 weeks later, spirally cut (Constantine 1965) and superfused in cascade (Vane 1964) with Krebs solution (37 °C, aerated with 95% O_2: 5% CO_2) at a flow rate of 2·5 ml min^{-1} over a rat stomach strip (Vane 1957) superfused with atropine (3×10^{-7} M), phentolamine (3×10^{-7} M), propranolol (7×10^{-7} M), mepyramine (3×10^{-7} M), methysergide (4×10^{-7} M), antagonists to cholinergic and adrenergic agents, histamine and 5-hydroxytryptamine respectively (Piper & Vane 1969), together with indomethacin (3×10^{-6} M) to prevent endogenous synthesis of PGs by the stomach strip (Eckenfels & Vane 1972). The changes in tone of both tissues were recorded isotonically with auxotonic levers (initial load 1 g for trachea and 2 g for rat stomach strip) using Harvard type 386 transducers connected to a Harvard type 350 linear chart recorder.

A dose response relationship of PGE_2 on the rat stomach strip was established and repeated throughout the experiment so that contractions of stomach strip by the effluent from the trachea could be bracketed. Differential superfusion bioassay had previously confirmed that E prostaglandins were the only biologically active AA metabolites of the cyclooxygenase pathway released from guinea-pig trachea (Grodzinska et al 1975; Burka & Paterson 1980a). The release of PGE-like material from the trachea in response to histamine, carbachol and AA was recorded in the absence and presence of drugs on the same tissue and the results were analysed by Student's *t*-test for paired data. The

* Correspondence.

† Sodium 7-[3-(4-acetyl-3-hydroxy-2-propylphenoxy)-2-hydroxypropoxy]-4-oxo-8-propyl-4*H*-chromene-2-carboxylate.