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COMMUNICATIONS

Possible differences in α-adrenoceptors in rat, guinea-pig and rabbit spleen

BARBARA HARPER, I. E. HUGHES*, JULIE STOTT, *Department of Pharmacology, Medical & Dental Building, University of Leeds, Leeds LS2 9JT, U.K.*

Evidence for subdivisions amongst α-adrenoceptors is strong only for a distinction between the pre- and post-synaptic α-adrenoceptors (Starke 1977) although there are many reports of possible differences between post-synaptic α-adrenoceptors in different tissues or different species (Sheys & Green 1972; Downie et al 1975; Holmgren & Nilsson 1975; Struyker-Boudier et al 1975; Duckles & Bevan 1976; Barker et al 1977; Harper et al 1978). The methods used in characterizing these receptors often involve measurement of the affinity constant or dissociation constant of agonists and/or antagonists for the receptor under appropriate conditions (Furchgott 1972). However, agonists and antagonists may bind to different states of the receptor (Changeux & Podleski 1968; Greenberg et al 1976) and different agonists may interact with different sites on the same receptor (Ruffalo et al 1977) which can give rise to difficulties in interpretation of the data obtained. We have therefore chosen to characterize the α-adrenoceptors in the rat, rabbit and guinea-pig spleen by measurement of the dissociation constant of a single agonist, noradrenaline, using an irreversible antagonist (phenoxybenzamine) as described by Furchgott (1966) and Mackay (1966). Spleen was chosen as the dissociation constant (K_a) of noradrenaline in this tissue in the rabbit has been reported previously (Sheys & Green 1972) and measurement of the dissociation constant of phentolamine in spleens from the cat, mouse and rabbit suggests that the α-adrenoceptors may not be identical in all these species (Sheys & Green 1972; Green & Fleming 1968; Ignarro & Titus 1968).

When appropriate, male rats (0·15–0·35 kg), rabbits (2·0–3·5 kg) and guinea-pigs (0·25–0·4 kg) were given reserpine (Sigma; 5 mg kg^{-1}; i.m. rabbits; i.p. others) 24 or 64 h before the animals were killed by a blow on the head. Splenic strips (1 mm wide and 30 mm long) were mounted in tissue baths at 36° C in physiological saline (NaCl 129·9, KCl 5·94, $CaCl_2$ 2·1, NaH_2PO_4 1·54, $NaHCO_3$ 25·0, glucose 11·1, sucrose 13·2 mM, gassed with 5% CO_2 in oxygen) and allowed to equilibrate for 20 min. The physiological saline was then replaced with physiological saline containing cocaine hydrochloride B.P. (3×10^{-5} M; to block uptake$_1$), β-oestradiol (Sigma; $7{\cdot}3 \times 10^{-7}$ M; to block uptake$_2$), (±)-propranolol hydrochloride ($1{\cdot}7 \times 10^{-6}$ M; ICI; to block β-adrenoceptors) and disodium ethylene-diamine-tetra-acetic acid (3×10^{-5} M; BDH, to prevent oxidative degradation of noradrenaline). When appropriate, iproniazid phosphate ($3{\cdot}6 \times 10^{-4}$ M; Sigma; to inhibit monoamine oxidase) was added to the bath, allowed to remain in contact with the tissue for 30 min and this tissue was then washed for 30 min. In all experiments U-0521 (3′-4′-dihydroxy-2-methyl propiophenone; 5·5 $\times 10^{-5}$ M; Upjohn; to block catechol-*O*-methyl-transferase) was added to the tissue bath 30 min before the determination of each cumulative dose-response curve to (−)-noradrenaline. Changes in length of the tissues in response to 2-fold increments in concentration of (−)-noradrenaline bitartrate (Sigma) were recorded isotonically (contact time 3–5 min; load 0·3 g rabbit; 0·2 g others) and tissues were washed by overflow. After determination of the second dose-response curve phenoxybenzamine hydrochloride ($0{\cdot}88$–$1{\cdot}17 \times 10^{-6}$ M for rat spleen; $0{\cdot}73$–$4{\cdot}4 \times 10^{-8}$ M others; SKF) was added. These concentrations of phenoxybenzamine were chosen so as to produce a satisfactorily large displacement of the $\log_{10}$ dose-response curve and were allowed to remain in contact with the tissue for 15 min. The tissue was then washed for 5 min, U-0521 added and a third dose-response curve to (−)-noradrenaline was determined 30 min later.

From $\log_{10}$ dose response curves before and after treatment with phenoxybenzamine (curves 2 and 3 respectively) equieffective concentrations of (−)-noradrenaline before (A) and after (A′) phenoxybenzamine treatment were obtained. Plots of 1/A against 1/A′ gave a linear relationship and the dissociation constant of noradrenaline was obtained from the relationship

$$K_a = (\text{slope}-1)/(\text{intercept on the } 1/A \text{ axis}).$$

The fraction of receptors remaining after phenoxybenzamine treatment was obtained from the relationship

$$\text{fraction remaining} = 1/\text{slope}.$$

The derivation of these relationships is well documented in the literature (Furchgott 1966; Mackay 1966).

Initial experiments showed that noradrenaline produced an equilibrium response in all three species after 3–5 min and the response showed no tendency to fade. Repeated determinations of $\log_{10}$ dose-response curves to (−)-noradrenaline showed that the second and third curves were similar but were often different in both slope

* Correspondence.

and position from the first curve. Only data from the second and third curves were therefore used in the calculation of K_a values.

Individual values for the dissociation constant of (−)-noradrenaline in spleens from the three species and under different experimental conditions are shown in Table 1. Two features of the results are notable. Firstly, the relative independence of the K_a values on the experimental conditions and secondly, the size of the difference between the mean K_a value calculated from the pooled data for the rat ($1{\cdot}75 \pm 0{\cdot}32 \times 10^{-5}$; mean ± s.e.m., n = 8) and those calculated for the rabbit ($8{\cdot}93 \pm 2{\cdot}01 \times 10^{-7}$; mean ± s.e.m.; n = 5) and the guinea-pig ($4{\cdot}57 \pm 0{\cdot}67 \times 10^{-7}$; mean ± s.e.m.; n = 4). The difference is some 20-fold between rat and rabbit and some 40-fold between rat and guinea-pig. Both these differences are significant statistically ($P < 0{\cdot}01$ in both cases) while the mean values obtained for rabbit and guinea-pig do not show any statistically significant difference ($P > 0{\cdot}1$).

In our hands two determinations of the K_a value for (−)-noradrenaline in rabbit spleen performed under the conditions detailed by Sheys & Green (1972) gave values ($11{\cdot}67$ and $8{\cdot}25 \times 10^{-7}$) which were very close to the mean value ($7{\cdot}38 \times 10^{-7}$) obtained by Sheys & Green themselves. In addition, this mean value did not differ significantly ($P > 0{\cdot}6$) from the mean value we obtained under our experimental conditions.

From the differences in the K_a values it might be concluded that the α-adrenoceptors in rat spleen are not identical to those in guinea-pig or rabbit spleen. The validity of this conclusion is however dependent on certain experimental criteria which must be fulfilled if meaningful comparisons of K_a values are to be made (Furchgott 1972).

Sufficient time was allowed for the response to each concentration of noradrenaline to reach an equilibrium value and only the second and third dose-response curves, which were reproducible, were used in the calculation of K_a values. An involvmement of β-adrenoceptors in the response to noradrenaline was eliminated by the use of propranolol at a concentration some 500 times the pA_2 of this antagonist and β-adrenoceptors should therefore be blocked even if they do contribute to the response to noradrenaline in rat, rabbit or guinea-pig spleen which seems unlikely (Ignarro & Titus 1968). The remaining criteria are primarily concerned with the elimination of all sites of loss of noradrenaline since differences in the sites of loss in the different species could account for differences in the K_a values. We have already demonstrated that ethylenediaminetetra-acetic acid will prevent the oxidative degradation of noradrenaline in physiological saline (Hughes & Smith 1978) and the concentration of cocaine used is known to be maximally effective against the uptake of noradrenaline in vas deferens at least (Hughes 1978). At the concentration used iproniazid is known to completely inhibit the action of monoamine oxidase even if this enzyme is

Table 1. Dissociation constant (K_a) for (−)-noradrenaline and percentage of α-adrenoceptors remaining after phenoxybenzamine treatment in rat guinea-pig and rabbit splenic strips. The results of individual determinations of the dissociation constant are shown and all experiments were performed in the presence of cocaine, propranolol, U-0521, β-oestradiol and disodium ethylenediaminetetra-acetic acid (see text). Treatment with iproniazid ($3{\cdot}6 \times 10^{-4}$ M) or reserpine (5 mg kg^{-1} 24 or 64 h before experiment) is indicated. The results from two determinations performed under the conditions described by Sheys & Green (1972) are also shown as is the mean value that they reported.

Species	Conditions: Reserpine	Conditions: Iproniazid	Noradrenaline K_a	Mean ± s.e. mean	Receptors remaining %
			$0{\cdot}68 \times 10^{-5}$		7·0
Rat	24 h	+	$1{\cdot}85 \times 10^{-5}$		5·0
			$1{\cdot}03 \times 10^{-5}$		18·8
			$0{\cdot}95 \times 10^{-5}$	$1{\cdot}75 \pm 0{\cdot}32 \times 10^{-5}$	19·0
Rat	64 h	+	$3{\cdot}23 \times 10^{-5}$	(n = 8)	2·3
			$2{\cdot}07 \times 10^{-5}$		6·9
Rat	—	—	$2{\cdot}78 \times 10^{-5}$		2·9
			$1{\cdot}43 \times 10^{-5}$		3·2
Rabbit	24 h	—	$13{\cdot}2 \times 10^{-7}$		13·1
			$6{\cdot}45 \times 10^{-7}$		3·9
			$14{\cdot}29 \times 10^{-7}$	$8{\cdot}93 \pm 2{\cdot}01 \times 10^{-7}$	9·7
Rabbit	—	—	$6{\cdot}33 \times 10^{-7}$	(n = 5)	7·8
			$4{\cdot}37 \times 10^{-7}$		28·0
Rabbit	Sheys & Green's conditions		$11{\cdot}67 \times 10^{-7}$	$9{\cdot}96 \times 10^{-7}$	34·1
			$8{\cdot}25 \times 10^{-7}$	(n = 2)	10·2
Rabbit	Sheys & Green's conditions		Sheys & Green's value	$7{\cdot}38 \pm 2{\cdot}47 \times 10^{-7}$ (n = 6)	
			$5{\cdot}59 \times 10^{-7}$		14·4
Guinea-pig	24 h	—	$3{\cdot}39 \times 10^{-7}$	$4{\cdot}57 \pm 0{\cdot}67 \times 10^{-7}$	38·0
			$3{\cdot}42 \times 10^{-7}$	(n = 4)	16·4
			$5{\cdot}88 \times 10^{-7}$		11·8

important as a site of loss for noradrenaline in a tissue where uptake$_1$ is already blocked (Furchgott 1955). Certainly the omission of iproniazid treatment made little difference to the K_a values obtained in the rat. The uptake$_2$ process for noradrenaline may not be completely inhibited by the concentration of β-oestradiol used (Kalsner 1969) but this concentration was chosen since higher concentration produced a sharp decrease in the responsiveness of the tissues. Kalsner has shown however that potentiation of the response to noradrenaline by uptake$_2$ blockers in the aortic strip was abolished in the presence of U-0521 (1×10^{-5} M). Furthermore, K_a values determined in aortic strips in the presence and absence of an uptake$_2$ blocker show only marginal differences (Besse 1975; Besse & Furchgott 1976) and it seems unlikely therefore that incomplete blockade of the uptake$_2$ process could account for a difference in K_a value of the size we have observed.

Reserpinization of the rabbits or rats made little difference to the K_a value and this is in agreement with the findings of Sheys & Green (1972) and would be expected since we know of no evidence to suggest that exogenous noradrenaline induces part of its response by a release of endogenous noradrenaline.

One final possibility is that the different concentration of phenoxybenxamine used in the rat from that used in the rabbit and guinea-pig has given an artifactual difference in the K_a value. However, Besse & Furchgott (1976) have shown that K_a values are essentially independent of the concentration of the irreversible antagonist used and furthermore, there was no consistent difference between the fraction of receptors remaining active after phenoxy benzamine treatment in the three species or any obvious relationship between the fraction of receptor remaining and the K_a obtained.

We would suggest therefore that the large difference between the dissociation constant for noradrenaline in rat spleen and that in rabbit and guinea-pig spleen is due to a difference in the α-adrenoceptors in these tissues.

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