J. Pharm. Pharmacol. 1986, 38: 902–906
Received May 7, 1986

Calculated and actual changes in β-adrenoceptor number associated with increases in rat and guinea-pig cardiac sensitivity

RUSSELL G. CHESS-WILLIAMS*, KENNETH J. BROADLEY† AND DESMOND J. SHERIDAN‡§

Cardiovascular Research Laboratory, Department of Pharmacology, Welsh School of Pharmacy, University of Wales Institute of Science and Technology, PO Box 13, and §Department of Cardiology, University of Wales College of Medicine, Cardiff CF4 4XN, UK

Chronic catecholamine depletion induced by reserpine pretreatment of rats, or 6-hydroxydopamine pretreatment of guinea-pigs, resulted in an enhanced sensitivity of isolated papillary muscles to isoprenaline. This hypersensitivity was accompanied by 1·41-(rats) and 1·52-fold (guinea-pigs) increases in the number of [^{3}H]dihydroalprenolol binding sites, without changes in binding affinity. An equation was derived for calculation of increases in receptor number. Application of this showed that substantially greater increases in receptor number were required (2·32- to 4·04-fold) to account for the degree of supersensitivity observed.

Chronic catecholamine depletion (Tenner et al 1982; Broadley et al 1984) or complete sympathetic ablation with 6-hydroxydopamine (Chess-Williams et al 1985) produces a post-synaptic potentiation of cardiac β-adrenoceptor-mediated responses. This increase in sensitivity occurs selectively at the β-adrenoceptor and does not occur at cardiac α- (Latifpour & McNeil 1984) or histamine receptors (Hawthorn & Broadley 1982; Chess-Williams et al 1985).

The precise mechanism of this increased sensitivity is unknown, but it is believed to reside at the β-adrenoceptor itself (Broadley et al 1984). An increase in β-adrenoceptor agonist affinity has been discounted for both reserpine and 6-hydroxydopamine-induced supersensitivity (Broadley et al 1984). The situation concerning an increase in β-adrenoceptor number however is less clear. For chronic reserpine pretreatment a number of studies have been reported which either support the concept of a proliferation of β-adrenoceptors (Tenner et al 1982; Latifpour & McNeill 1984) or refute it (Hawthorn & Broadley 1982; Torphy et al 1982). A few studies support the idea of an increase in β-adrenoceptor number following chronic 6-hydroxydopamine pretreatment (Nomura et al 1980; Yamada et al 1980).

This study examines [^{3}H]dihydroalprenolol ([^{3}H]DHA) binding to cardiac membranes prepared from control and catecholamine-depleted animals and attempts to correlate the radioligand binding results with changes in the pharmacological sensitivity of isolated cardiac tissues.

* Present addresses: Department of Pharmacology and Therapeutics, University of Liverpool. ‡ Department of Cardiology, St Mary's Hospital Medical School, London.
† Correspondence.

MATERIALS AND METHODS

Pretreatment regimes

Male Wistar rats (250 g) were pretreated with reserpine hydrochloride (1 mg kg^{-1} daily) by intraperitoneal injection for 7 days. Guinea-pigs (450–550 g) were pretreated with 6-hydroxydopamine administered by intracardiac injection under halothane/nitrous oxide anaesthesia. Animals received 10 mg kg^{-1} of 6-hydroxydopamine on day 1, 50 mg kg^{-1} on day 2 and 100 mg kg^{-1} on days 7, 8, 14 and 15. Experiments were performed on day 20.

Isolated tissues

Rats (male, Wistar) and guinea-pigs (male, Dunkin-Hartley) were killed by a blow to the head and exsanguinated. The hearts were rapidly removed and papillary muscles set up under 0·8 g resting tension in a Krebs-bicarbonate solution (composition in mM: NaCl 118·4, KCl 4·7, $CaCl_2.2H_2O$ 1·9, $NaHCO_3$ 25, $MgSO_4.7H_2O$ 1·2, glucose 11·7, KH_2PO_4 1·2) gassed with 5% CO_2 in O_2 at 32 °C. Tissues were field stimulated at 1 Hz (threshold voltage + 50%; 5 ms pulse width) with bipolar ring electrodes, and isometric developed tension recorded. Experiments were performed in the presence of metanephrine (10 μM) to inhibit extraneuronal uptake. At this concentration, the β-adrenoceptor antagonist properties of metanephrine would have been negligible; they only become apparent at 100 μM (Kenakin 1981).

Following a 30 min equilibration period, a cumulative concentration–response curve for isoprenaline was established. The increases in developed tension at each concentration were expressed as a percentage of the maximum increase and individual EC50 values were calculated.

Radioligand binding

After the removal of papillary muscles, the remaining ventricular tissue was assayed for [^{3}H]dihydroalprenolol ([^{3}H]DHA) binding. The ventricular tissue was homogenized in 50 mM Tris HCl buffer (pH 7·4) using a glass Teflon homogenizer. The homogenate was passed through muslin (4 layers) and centrifuged at 1000*g* for 10 min in an MSE chillspin centrifuge. The supernatant was recentrifuged in an MSE S/S 65 centrifuge at 35 000*g* for 10 min. The pellet was resuspended in 5 ml buffer and recentrifuged at 35 000*g* for 10 min to wash the membranes. The membrane fraction was washed 3 times in this manner before finally suspending in 3 ml buffer and assaying for [^{3}H]DHA binding. Saturation curves for [^{3}H]DHA binding at concentrations of 0·5, 1, 2, 4, 8 and 16 nM were obtained at 32 °C with nonspecific binding determined by displacement with 200 μM isoprenaline.

Statistical analysis

Geometric mean isoprenaline EC50 values with 95% confidence limits were calculated. Differences in tissue sensitivity between control and catecholamine-depleted animals were analysed by performing Student's unpaired *t*-test on individual logarithmic EC50 values. In radioligand binding experiments, the maximum number of binding sites (B_m) and the dissociation constants (K_D) of [^{3}H]DHA binding were calculated by Scatchard analysis of saturation curves, and differences between groups examined with Student's *t*-test.

Drugs used

(−)-Isoprenaline tartrate dihydrate (Ward Blenkinsop) was kindly supplied as a gift. (±)Metanephrine hydrochloride, 6-hydroxydopamine hydrobromide, and reserpine hydrochloride were obtained commercially (Sigma). [^{3}H]Dihydroalprenolol (77 Ci mmol^{-1}) was obtained from the Radiochemicals Centre, Amersham. Reserpine was dissolved in 20% ascorbic acid and 1 M NaOH with a final pH of 5·0.

RESULTS

Tissue sensitivity

Reserpine pretreatment of rats resulted in a leftward shift of the isoprenaline concentration–response curves (Fig. 1A). The EC50 value fell significantly from the control value of 18·6 (8·2–42·0) to 4·3 (2·8–6·7) nM in catecholamine-depleted tissues. In tissues from reserpine-pretreated rats, both the resting (1·06 ± 0·27 g) and maximal developed tension (1·66 ± 0·35 g) were greater than control values (0·67 ± 0·10 and 1·26 ± 0·16 g), although the difference was only significant ($P < 0·05$) for resting developed tension.

Pretreatment of guinea-pigs with 6-hydroxydopamine resulted in a similar leftward shift of the isoprenaline concentration–response curves of isolated papillary muscles (Fig. 1B). The EC50 value fell significantly ($P < 0·05$) from 5·2 (4·1–6·6) to 2·0 (1·2–3·3) nM for tissues from pretreated animals. Resting and maximal developed tensions were similar in both groups of tissues (0·46 ± 0·09 and 1·57 ± 0·25 g for control and 0·32 ± 0·11 and 1·57 ± 0·37 g for catecholamine-depleted tissues, respectively).

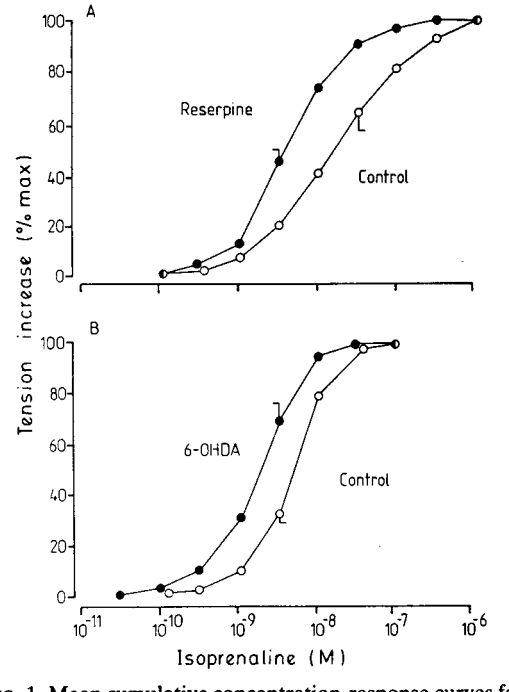

FIG. 1. Mean cumulative concentration-response curves for the positive inotropic responses to isoprenaline of papillary muscles obtained from control animals (open symbols) and those chronically depleted of catecholamines (solid symbols). Catecholamine depletion was induced by: A, reserpine pretreatment of rats (1 mg kg^{-1} daily for 7 days i.p.) and B, 6-hydroxydopamine (6-OHDA) pretreatment of guinea-pigs (460 mg kg^{-1} given in divided intracardiac injections over 20 days). Experiments were performed in the presence of metanephrine (10 μM). Increases in tension are plotted as a percentage of the maximum response and each point is the mean value (n ≥ 4) with the s.e.m. at a mid-curve point.

[^{3}H]Dihydroalprenolol binding

The dissociation constants (K_D) for the binding of [^{3}H]DHA to ventricular membranes were similar in all tissues examined. However, the number of [^{3}H]DHA binding sites (B_m) varied considerably, being significantly ($P < 0.001$) greater in the guinea-pig than in the rat ventricle (Table 1). Chronic catecholamine depletion by 6-hydroxydopamine or reserpine resulted in a significant ($P < 0.05$) increase in the number of [^{3}H]DHA binding sites in both the guinea-pig (1·52-fold) and rat (1·41-fold). However, there was no change in the dissociation constant (Table 1).

DISCUSSION

Chronic pretreatment of rats with reserpine and of guinea-pigs with 6-hydroxydopamine induced supersensitivity to the inotropic effects of isoprenaline. This confirms previous reports of an increase in cardiac sensitivity to β-adrenoceptor agonists after chronic catecholamine depletion (see Broadley et al 1984). There was an increase in basal developed tension after reserpine pretreatment which agrees with previous studies (Broadley & Lumley 1977; Tenner et al 1982; Torphy et al 1982) and has been interpreted to indicate a change in the excitation–contraction coupling (Tenner et al 1982). However, the consensus is that the supersensitivity to β-adrenoceptor agonists is additional to any change in coupling and in the present study it was associated with an increase in β-adrenoceptor binding sites. Other studies have also demonstrated increases in the number of cardiac β-adrenoceptor binding sites after pretreatment with reserpine (Tenner et al 1982, 1984; Latifpour & McNeill 1984) or 6-hydroxydopamine (Nomura et al 1980; Yamada et al 1980). However, two reports, including one from our laboratories, have failed to demonstrate an increase in these binding sites after reserpine pretreatment (Hawthorn & Broadley 1982; Torphy et al 1982). An explanation for the difference between these and the present study is not readily available. The reserpine-induced changes may be species-dependent since in this laboratory guinea-pigs failed to reveal a significant receptor increase (Hawthorn & Broadley 1982), whereas in the present study rats did. Additionally, the time course and dosage of reserpine pretreatment may be of relevance, since Tenner et al (1984) have shown an increase in receptor number in rat ventricular tissue after 7 days but not after 1 or 3 days of treatment.

Few studies have examined receptor number simultaneously with tissue sensitivity and to our knowledge none has attempted to correlate the two measurements. Although an increase in receptor number has been detected and linked to the enhanced tissue sensitivity, it does not necessarily explain it and may only partially contribute to the change in cardiac sensitivity observed. We have therefore attempted to correlate changes in receptor number with tissue responsiveness by deriving an equation to relate these two measurements.

Assuming that the interaction between receptor [R] and agonist [A] follows a simple bimolecular reaction, the increase in receptor number required to explain a decrease in agonist EC50 value from $[A_1]$ to $[A_2]$ can be calculated as follows:

By the Law of Mass Action, for control tissues

$$\frac{RA_1}{RT_1} = \frac{[A_1]}{K_A + [A_1]}$$

while for catecholamine-depleted tissues

$$\frac{RA_2}{RT_2} = \frac{[A_2]}{K_A + [A_2]}$$

where RA = receptor occupancy at EC50, RT = receptor occupancy of the total functional receptors and K_A = dissociation constant of isoprenaline for the cardiac β-adrenoceptor.

Table 1. Dissociation constants (K_D) and maximum number of binding sites (B_m) for [^{3}H]DHA binding to cardiac membranes prepared from control and catecholamine-depleted guinea-pigs[a] and rats[b].

	Control ventricle			Catecholamine-depleted			
	K_D (nM)	n	B_m (fmol mg^{-1} protein)	K_D (nM)	n	B_m (fmol mg^{-1} protein)	B_m ratio
Guinea-pig	3·4 ± 0·8	6	43·1 ± 6·7	4·2 ± 0·3	6	65·4 ± 8·3*	1·52
Rat	1·6 ± 0·5	6	25·9 ± 3·1	2·4 ± 0·3	6	36·6 ± 3·2*	1·41

Mean values (± s.e.m.) are shown and a significant difference between values for control and depleted animals, as determined by Student's unpaired *t*-test, is indicated by $*P < 0.05$.

[a] Guinea-pigs were pretreated with 6-hydroxydopamine (460 mg kg^{-1} given in divided intracardiac injections over 20 days).

[b] Rats received reserpine (1 mg kg^{-1} daily for 7 days i.p.).

We can assume that there is no change in K_A between controls and depleted tissues and this is justified by previous studies (Hawthorn & Broadley 1982). Then equal agonist receptor occupancy can be assumed to occur with equal responses in both control and depleted tissues

$$RA_1 = RA_2$$

therefore

$$\frac{[A_1]RT_1}{K_A + [A_1]} = \frac{[A_2]RT_2}{K_A + [A_2]}$$

rearranging:

$$\frac{RT_2}{RT_1} = \frac{[A_1](K_A + [A_2])}{[A_2](K_A + [A_1])}$$

where RT_2/RT_1 is the ratio of total receptor number in supersensitive and control tissues.

This equation allows the calculation of the change in functional receptors required to produce a given change in tissue sensitivity as measured by a fall in EC50 value from $[A_1]$ to $[A_2]$. This assumes that only a change in receptor number occurs and that the K_A value for isoprenaline remains constant and is known. Dissociation constants for isoprenaline have been determined for cardiac β_1-adrenoceptors by pharmacological and radioligand binding techniques. They are known to vary with the bathing temperature (Broadley & Williams 1983), the ionic composition of the medium and the presence of GTP (McPherson et al 1985). Values ranging from about 25 nM for both binding (Hawthorn & Broadley 1982) and pharmacological determinations (Buckner et al 1978; Broadley & Nicholson 1981; Broadley & Williams 1983) to 200 nM for binding (McPherson et al 1985) have been calculated. If these values are substituted in the equation, the increase in receptor number required to account for the change in sensitivity due to catecholamine depletion by 6-hydroxydopamine ranges from 2·32- to 2·56-fold (132–156% increase). The corresponding values for reserpine-induced supersensitivity are 2·91- to 4·04-fold (i.e. 191–304% increase in receptor number). The values are far in excess of the receptor increases actually found by radioligand binding experiments which were 1·52-fold (52%) and 1·41-fold (41%), respectively, for the 6-hydroxydopamine- and reserpine-pretreated animals. This suggests that although an increase in β-adrenoceptor number is associated with depletion-induced supersensitivity, it cannot fully explain the change in sensitivity.

It is possible that [^{3}H]DHA may bind to β-adrenoceptors which are non-functional cardiac β-adrenoceptors. This limitation will, of course, always apply where attempts to correlate receptor binding with tissue sensitivity are made. An advantage of the present calculation is that equal responses between groups are compared (a null method), hence no assumption is made regarding the relationship between receptor occupation and response.

Few other studies have examined changes in receptor number and tissue sensitivity simultaneously in tissue from the same animal. Tenner et al (1982) observed a 7·5-fold change in EC50 of isoprenaline on rabbit papillary muscles following chronic reserpine pretreatment. The increase in [^{3}H]DHA binding which accompanied this supersensitivity was however only 29·6% (1·29-fold). By application of the present equation to their results, an increase in binding sites of between 2·45- and 5·53-fold would have been required if a proliferation of receptors was the sole mechanism of the sensitivity change. Similarly, reserpine pretreatment produced a 3-fold shift of isoprenaline dose-response curves of working guinea-pig hearts, yet this was associated with an increase in β-adrenoceptor binding sites of only 35·8% (1·36-fold) (Latifpour & McNeill 1984). A 2·78- to 2·97-fold increase would be required to produce such an alteration in sensitivity.

This consistent failure of the calculated increase in receptor number to match the value determined by radioligand binding indicates that the assumptions made in deriving the equation were invalid. Thus a simple receptor proliferation is not the sole mechanism for the depletion-induced supersensitivity and additional factors must contribute to the sensitivity change. The K_A value does not appear to alter (Hawthorn & Broadley 1982), however, a possible change in coupling between receptor and adenylate cyclase or between stimulus and response must be considered. It is interesting that the resting developed tension was greater in depleted tissues than controls, although the difference was significant only with reserpine. A similar increase in resting developed tension has been reported by other investigators (Tenner et al 1982; Torphy et al 1982) and may indicate an enhanced utilization of calcium by cardiac cells as suggested by Tenner et al (1982). On the other hand, responses to forskolin, a direct stimulant of adenylate cyclase, are not affected by chronic reserpine pretreatment (Hawthorn et al 1985). This suggests that the additional mechanism responsible for the increase in β-adrenoceptor sensitivity may lie at a stage preceding cAMP generation, such as the coupling mechanism between the β-adrenoceptor and adenylate cyclase.

In conclusion, it appears that the increase in β-adrenoceptor sensitivity following chronic catecholamine depletion cannot be explained by an increase in β-adrenoceptor number alone, and the nature of the additional mechanism(s) remains to be established.

Acknowledgements

This work was supported by a grant from the Medical Research Council. We are grateful to the companies concerned for generous gifts of drugs.

REFERENCES

Broadley, K. J., Lumley, P. (1977) Br. J. Pharmacol. 59: 51–60

Broadley, K. J., Nicholson, C. D. (1981) Ibid. 72: 635–643

Broadley, K. J., Williams, R. G. (1983) Ibid. 79: 517–524

Broadley, K. J., Williams, R. G., Hawthorn, M. H., Grassby, P. F. (1984) Meth. Find. Exp. Clin. Pharmacol. 6: 179–186

Buckner, C. K., Torphy, T., Costa, D. J. (1978) Eur. J. Pharmacol. 47: 259–271

Chess-Williams, R. G., Grassby, P. F., Culling, W., Penny, W., Broadley, K. J., Sheridan, D. J. (1985) Naunyn-Schmiedeberg's Arch. Pharmacol. 329: 162–166

Hawthorn, M. H., Broadley, K. J. (1982) Ibid. 320: 240–245

Hawthorn, M. H., Chess-Williams, R. G., Grassby, P. F., Broadley, K. J. (1985) J. Auton. Pharmacol. 5: 231–239

Kenakin, T. P. (1981) Naunyn-Schmiedeberg's Arch. Pharmacol. 316: 89–95

Latifpour, J., McNeill, J. H. (1984) Can. J. Physiol. Pharmacol. 62: 23–26

McPherson, G. A., Molenaar, P., Malta, E., Raper, C. (1985) Br. J. Pharmacol. 84: 84P

Nomura, Y., Kajiyama, H., Segawa, T. (1980) Eur. J. Pharmacol. 66: 225–232

Tenner, T. E. Jr., Mukherjee, A., Hester, R. K. (1982) Ibid. 77: 61–65

Tenner, T. E., Walthall, E. B., Ramanadham, S. (1984) Proc. West. Pharmacol. Soc. 27: 5–9

Torphy, T. J., Westfall, D. P., Fleming, W. W. (1982) J. Pharmacol. Exp. Ther. 223: 332–341

Yamada, S., Yamura, H. I., Roeske, W. R. (1980) Mol. Pharmacol. 18: 185–192