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α-Adrenoceptors of aortae from genetically hypertensive rats: reaction with 2-halogenoethylamines

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The responses of aortic rings from adult spontaneously hypertensive and Carworth normotensive Wistar rats to noradrenaline were compared. The former developed slightly more tension at low, and markedly less tension at high concentrations ($>10^{-8}$M) of noradrenaline. The α-adrenoceptors of these tissues were probed using 2-halogenoethylamines to compare quantitatively α-adrenoceptors in normotensive and hypertensive tissue. The kinetics of the recovery of response to noradrenaline from the short-lived antagonism produced by *NN*-dimethyl-2-bromo-2-phenylethylamine were identical in aortae from both strains of rat. The rate of recovery of response was the same as the rate of loss of tritium from the tissues, suggesting the absence of spare receptors in rat aorta. An estimation of the number of α-adrenoceptors in aortic tissue did not show a difference between normotensive and hypertensive tissues. Evidence is presented that 2-halogenoethylamines react at two kinetically distinguishable sites in rat aorta. The rates of return of response to noradrenaline after receptor blockade with *N*-(2-bromoethyl)-*N*-ethyl-*N*-1-naphthylmethylamine (SY-28) in tissues pretreated with *NN*-dimethyl-2-bromo-2-phenylethylamine were the same in aortic smooth muscle from both strains of rat. No significant difference was found in the reactions of 2-halogenoethylamine α-adrenoceptor antagonists in aortic tissue from either strain of rat.

Somlyo & Somlyo (1970), reviewing hyper-responsiveness of blood vessels in hypertensive animals, concluded that vascular hyper-reactivity was generally associated with hypertension. The conflicting results (Bandick & Sparks, 1970; Bohr & Sitrin, 1970; Clineschmidt, Geller & others, 1970; Haeusler & Haefely, 1970; McGregor & Smirk, 1970; Smirk, 1970; Finch, 1971; Kalsner, Ayitey-Smith & Ling, 1971; Nicholas, 1971; Overbeck, Swindall & others, 1971) are probably due to varying choice of animals, vascular tissue, methods of measuring muscle responsiveness and of inducing hypertension.

We used spontaneously hypertensive Wistar rats (SHR; see Okamoto, 1969) from a strain developed by Okamoto & Aoki (1963). Okamoto, Hazama & others (1966) and Haeusler & Haefely (1970) found that the vascular muscle of these rats was hyper-reactive to noradrenaline but Clineschmidt & others (1970) did not.

A potential method for differentiating α-adrenoceptors is to determine rate of recovery of tissue response after irreversible receptor blockade with *NN*-dimethyl-2-bromo-2-phenylethylamine, DMPEA (Moran, Triggle & Triggle, 1969; Janis & Triggle, 1971a). This, like the isomer ratio technique (Patil, 1969), has the theoretical advantage that drug diffusion to and from the receptor should not affect the values

obtained. Janis & Triggle (1971a) suggested that the kinetics of recovery of response from DMPEA blockade may be sensitive to subtle differences in the α-adrenoceptors of various tissues. DMPEA can be used to detect a second site of 2-halogenoethylamine interaction in smooth muscle (Moran, Swamy & Triggle, 1970; Janis & Triggle, 1971b; Swamy & Triggle, to be published) and to estimate the concentration of α-adrenoceptors (May, Moran & others, 1967). We have set out to determine whether differences in aortic α-adrenoceptors of SHR and Wistar normotensive rat could be detected using 2-halogenoethylamines.

METHODS

Animals and preparation

The male f22 SHR were of the strain developed from Japanese Wistar rats (Okamoto & Aoki, 1963); controls were male Carworth Farms normotensive (CFN) Wistar rats. The animals were 24–32 weeks old, had blood pressures of 211 ± 17 and 127 ± 6 mm Hg and weighed 350 ± 20 and 455 ± 44 g (mean ± s.d.) for 14 SHR and 12 CFN rats respectively. Systolic blood pressures were recorded from the tail of prewarmed unanaesthetized rats with a pneumatic pulse transducer and a Physiograph Four-A (E. & M. Instrument Company, Houston, Texas). The average of the three lower of five readings obtained was recorded as the blood pressure for each rat.

Animals were decapitated, the thoracic aortae excised immediately and placed in Krebs-bicarbonate solution (composition m mol: NaCl, 118; KCl, 4·7; $CaCl_2$, 2·5; KH_2PO_4 1·2; $MgSO_4$, 1·2; $NcHCO_3$, 12·5; dextrose, 11·1; EDTA, 0·01, made up in double distilled water and maintained at 37° and aerated with O_2:CO_2 95:5), and cleaned of fat and surrounding connective tissue. One to three adjacent segments 4 mm wide were cut from each aorta (using two parallel surgical blades separated by a plastic block) and suspended between two stainless steel hooks (Wohl, Hausler & Roth, 1967). Contractions were recorded isometrically with Grass FT 03C force-displacement transducers and a Model 5D Grass Polygraph. After exposure to the bath fluid for 15 min, 2 g of tension was maintained on each segment which was allowed to equilibrate for 2 h before noradrenaline was added. The bath solutions were changed at least once every 15 min (except in radiochemical experiments).

Fresh drug solutions were used for each experiment. (–)-Noradrenaline (Sigma Chemical Company, St. Louis, Missouri) free base was solubilized with dilute hydrochloric acid and diluted with normal saline containing 0·05% sodium metabisulphite. *NN*-Dimethyl-2-bromo-2-phenylethylamine (DMPEA) was made up in saline at room temperature and immediately placed on ice. *N*-(2-Bromoethyl)-*N*-ethyl-1-naphthylmethylamine hydrobromide (SY-28) was dissolved in saline at 37° and kept 20 min before use.

Dose-response curves to noradrenaline

One or more segments from a normotensive rat was paired with segments from a SHR. When more than one segment from the same animal was used, the results from those segments were averaged. After the equilibration period, noradrenaline solutions were added cumulatively to the bath until maximum response was obtained. Only one dose-response curve was obtained from each segment.

Recovery of response after 2-halogenoethylamine treatment

After the maximum tissue response to noradrenaline (10^{-5}M) became stable, one segment from the aorta of each rat was treated with 6×10^{-6}M DMPEA for 5 min, thoroughly washed and challenged with noradrenaline according to the times in Fig. 2. When the response to noradrenaline had recovered to its plateau after 2–3 h, the DMPEA treated and untreated segments from the same aorta were exposed to SY-28 (8×10^{-7}M/5 min), thoroughly washed and challenged with noradrenaline at the times in Fig. 2.

Radiochemical kinetics and the concentration of α-adrenoceptors

Two segments from the aorta of each strain of rat were treated simultaneously with [^{3}H]DMPEA (6×10^{-6}M for 5 min), the % response to noradrenaline determined and the pharmacological recovery of response was followed in one segment from each aorta as described above. The rate of tritium loss from the other tissues was determined by taking 500 μl aliquots of the bathing solution at 10 min intervals and counting by liquid scintillation (Packard Tri Carb, model 3374); internal standards were used for quench correction (Rogers & Moran, 1966). A minimum of 10 000 counts was made. The data were plotted according to Rose (1964), as previously described (May & others, 1967), and the first-order rate constant determined.

In other experiments, three aortic rings were obtained from each animal. One segment was exposed to $1{\cdot}6 \times 10^{-6}$M SY-28 for 5 min, washed and the % response to noradrenaline determined. The segment was washed again and then immediately treated with [^{3}H]DMPEA simultaneously with the other two segments. The SY-28 pretreated tissue and a second tissue were used to measure tritium loss and the third tissue for recovery of response as described above. From a significant difference in the (counts/min)/mg dry wt calculated for the bath containing untreated and that for SY-28 treated tissues, an upper estimate of the number of α-adrenoceptors (May & others, 1967) can be made.

The tissues were dried to constant weight at 105° and solubilized in 1 ml of Soluene 100 (Packard) by shaking for 24 h at 55°. The scintillation fluid for tissues and bath samples had the following composition: 900 ml dioxane, 100 ml toluene, 60 g naphthalene, 10 g PPO, 0·5 g dimethyl POPOP. The tissues were counted as described above.

Mean values are given with their standard errors and were compared by Student's *t*-test. Differences with P values of 0·05 or less were considered significant.

RESULTS

Noradrenaline dose-response curves

The threshold concentration of noradrenaline was 10^{-10} to 3×10^{-10}M for aortic rings from the normotensive rats, but rings from SHR always produced significant tension at the lowest concentration (Fig. 1A) and significantly more tension than did the controls at 10^{-10}–10^{-9}M but less at 10^{-8}–10^{-5}M. Plots of response as a % of the maximum response clearly show the greater sensitivity of the SHR aorta to the lower concentrations of noradrenaline (Fig. 1B). Clineschmidt & others (1970) have shown that aortic strips from the CFN rats develop greater maximum tension that that of NIH Wistar normotensive rats. The dried aortic rings weighed $1{\cdot}60 \pm 0{\cdot}03$ mg

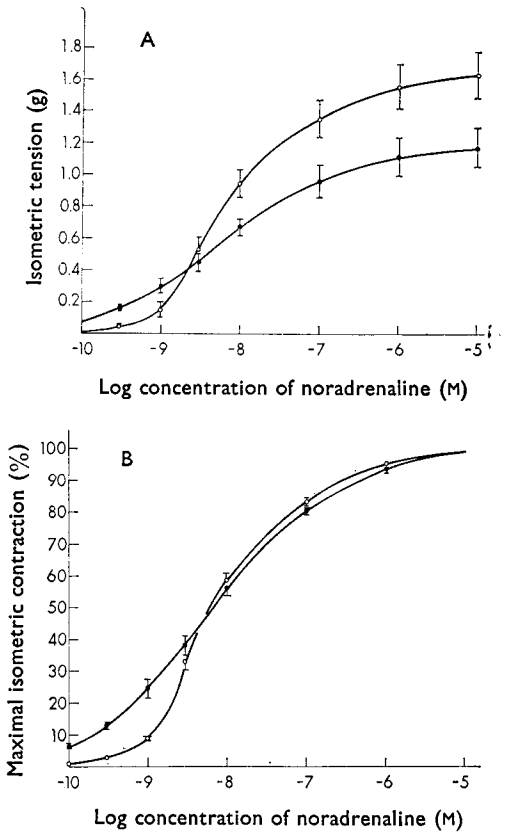

FIG. 1A. Isometric tension produced by aortic rings in response to the cumulative addition of noradrenaline.

B. Dose-response curves for noradrenaline calculated from the % maximal isometric contraction of each aortic ring used to obtain Fig. 1A.

Segments were obtained from eight spontaneously hypertensive (●) and ten normotensive (CFN) rats (○).

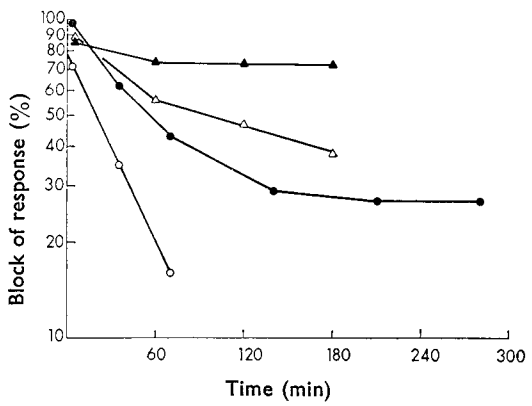

FIG. 2. Representative plots of the recovery of response to noradrenaline (10^{-5}M) after aortic rings were treated with the following 2-halogenoethylamines: *NN*-dimethyl-2-bromo-2-phenylethylamine (DMPEA) (6×10^{-6}M/5 min) observed (●) and corrected for plateau (O); SY-28 (8×10^{-7}M/5 min) (▲); SY-28 (8×10^{-7}M/5 min) 5–6 h after DMPEA (6×10^{-6}M/5 min) (△).

(n = 14), 1·52 ± 0·04 mg (n = 16) from SHR and CFN rats respectively; these values are not significantly different.

Recovery of response to noradrenaline after 2-halogenoethylamines

Representative plots for the recovery of response to noradrenaline for aortic tissue of normotensive rats after exposure to 2-halogenoethylamines are shown in Fig. 2. In all tissues 6×10^{-6}M for 5 min DMPEA produced a 90–98% block of response and recovery reached a plateau in 2–3 h. When the kinetics were corrected for the plateau, a first-order plot was obtained (Fig. 2). The times for 50% recovery of response to noradrenaline after [^{3}H]DMPEA treatment ($t_{1/2}$) were for SHR 31·7 ± 2·9 (n = 9) and for CFN 31·2 ± 1·7 (n = 5)—identical results.

Typical response recovery plots after exposure of aortic rings to SY-28 (8×10^{-7}M) for 5 min, which produced 85–99% block to noradrenaline, show a faster recovery rate for tissue pretreated with DMPEA 5–6 h before SY-28 treatment than tissues not pretreated (Fig. 2). The rate was constant after 1 h and the % recovery from 1 to 3 h after SY-28 was washed from the bath is the same for SHR and control tissues (21·6 ± 4·9, n = 8 and 24·2 ± 2·9, n = 6, respectively) and significantly different from tissues not pretreated (6·8 ± 1·3, n = 5 and 1·0 ± 0·8, n = 5, respectively). The average % block of response was not significantly different in control and DMPEA-pretreated tissues.

Kinetics of the loss of tritium from aortic rings and estimation of the number of α-adrenoceptors

Tissues treated with 6×10^{-6}M [^{3}H]DMPEA for 5 min were blocked 90–98%. The data were plotted according to Rose (1964) as described by May & others (1967) and the $t_{\frac{1}{2}}$'s for radioactivity appearing in the bath fluid was found to be 34·4 ± 6·7 (n = 5) and 39·4 ± 6·3 (n = 5) for the SHR and CFN respectively. These values are not significantly different from the $t_{\frac{1}{2}}$'s (above).

We attempted to estimate the number of α-adrenoceptors, but the number of molecules of [^{3}H] label ($\times 10^{12}$)/mg tissue dry weight leaving the control and SY-28 pretreated tissues did not differ significantly (6·2 ± 0·9, n = 6 and 5·4 ± 0·5, n = 3 for SHR; 6·9 ± 1·1, n = 5 and 6·1 ± 0·7, n = 3 for CFN rat aortae, respectively). The difference is $0{\cdot}8 \times 10^{12}$ molecules/mg tissue dry weight in each case. The tissues were removed from the bath 10 h after DMPEA exposure and the amount of label remaining was determined. Again SY-28 pretreatment ($1{\cdot}6 \times 10^{-6}$/5 min) failed to significantly reduce the uptake of radioactivity (5·4 ± 0·5, n = 3 and 6·1 ± 0·7, n = 3 for SHR and CFN respectively). The number of molecules of DMPEA bound to the SHR tissues not treated with SY-28 ($6{\cdot}1 \pm 0{\cdot}5 \times 10^{12}$/mg dry weight tissue, n = 9) did not differ significantly from that of Wistar normotensive tissues ($5{\cdot}5 \pm 0{\cdot}5 \times 10^{12}$, n = 8), nor from the amount of radioactivity leaving the tissues.

DISCUSSION

Clineschmidt & others (1970) concluded that adrenoceptors mediating aortic contraction are similar in SHR and normotensive rats. From the similarity in the apparent dissociation constants for phentolamine among various tissues from different species, they predicted that α-adrenoceptors in arteriolar muscle would not differ from those in aortic muscle. The kinetics of recovery of response from DMPEA provides a possible alternative technique for comparison of α-adrenoceptor structure

(Janis & Triggle, 1971a). Furthermore, DMPEA provides a means to show that many 2-halogenoethylamines (dibenamine, SY-28, etc.) have at least two sites of interaction where they modify α-adrenoceptor responses (Moran & others, 1970; Swamy & Triggle, 1972): one appears to be a Ca^{2+} binding/mobilization site specifically associated with α-adrenoceptor activation. Since a derangement of Ca^{2+} function in SHR may produce adrenergic hyper-reactivity, we attempted to compare the α-adrenoceptor-mediated responses of mature SHR to determine whether, relative to normotensive controls, differences were present that might be involved in the maintenance of, or caused by, hypertension.

The noradrenaline dose-response curves obtained with aortic rings from adult SHR and CFN rats (Fig. 1A) generally agree with the results of Spector, Fleisch & others (1969) and those of Clineschmidt & others (1970) from aortic strips of young rats of the same strains. We observed a slight but significant hyper-reactivity in SHR aorta at the lowest doses (10^{-10}–10^{-9}M) of noradrenaline (Fig. 1A,B). Clineschmidt & others (1970) reported that aortic strips from young SHR developed more tension than those from NIH Wistar normotensive rats at the six lowest doses studied ($<$ than 10^{-8}M), though the differences were not statistically significant. The problems of obtaining appropriate control animals (Clineschmidt & others, 1970) and of estimating the concentration of noradrenaline at the receptor *in vivo* make it difficult to associate the maintenance of hypertension with the slight hyper-responsiveness which we observed. The greater tension developed by the CFN rats was not associated with a larger tissue mass.

A comparison of the kinetics of recovery of response to noradrenaline after DMPEA blockade showed no differences between SHR and CFN aortic segments, but in these tissues the recovery of response was not a smooth function but showed an obvious plateau (Fig. 2) in contrast to results with rabbit aorta (May & others, 1967; Janis & Triggle, 1971b) and rat vas deferens (Moran & Triggle, 1970; Swamy & Triggle, 1972) where a completely linear recovery of response was obtained. A first-order plot for recovery of response in the rat aorta was, however, obtained (Fig. 2) by compensation of the experimental plot for the plateau.

DMPEA, when administered before long-acting antagonists like phenoxybenzamine and SY-28, significantly decreased the duration, but not the degree, of antagonism with rat vas deferens (Moran & Triggle, 1970; Swamy & Triggle, 1972) and rabbit aorta (Janis & Triggle, 1971b). We have proposed that there may be two sites at which 2-halogenoethylamines exert antagonism and that these may be distinguished by different durations of antagonism as shown after DMPEA pretreatment. Since the effect of DMPEA may be duplicated by a number of Ca^{2+}-competing species, including local anaesthetics and diazoxide, we have suggested that these sites may represent noradrenaline recognition and Ca^{2+} binding components of the α-adrenoceptor. The results with the rat aorta show that after DMPEA pretreatment and allowing the response to recover to its maximum extent, the recovery from blockade by subsequently administered SY-28 is significantly faster than in untreated controls, in tissues from both normotensive and hypertensive species and that no significant difference in this respect exists between the tissues.

No differences were apparent between the rates of loss of [^{3}H]label from SHR and CFN aortae, and the radiochemical $t_{1/2}$ times were identical to the corrected pharmacological $t_{1/2}$ values, which suggests the absence of any receptor reserve in this α-adrenoceptor system, as reported for other systems (Lewis & Miller, 1966; May

& others, 1967; Moran, Triggle & Triggle, 1969; Yong & Marks, 1969; Triggle, 1971).

The above data and our attempt to estimate the number of α-adrenoceptors in aortae from CFN and SHR rats by determining the amount of [^{3}H]label leaving the [^{3}H]DMPEA blocked tissues during the recovery of pharmacological response were both severely complicated by the fact that pretreatment of the tissues with a blocking concentration of SY-28 produced no significant reduction in the amount of label lost from the tissue. Unless SY-28 and DMPEA exert their antagonism at totally distinct sites, it must be concluded that much of the [^{3}H]DMPEA bound and lost from these tissues represents non-receptor material. Our use of 2-halogenoethylamines as receptor probes has not shown any difference in the number or characteristics of α-adrenoceptors in aortae from normotensive and hypertensive rats, but such differences may exist and be beyond the sensitivity and specificity of our techniques.

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REFERENCES

BANDICK, N. R. & SPARKS, H. V. (1970). *Am. J. Physiol.*, **219**, 340–344.

BOHR, D. F. & SITRIN, M. (1970). *Circulation Res.*, **27**, *Suppl.* 11, 83–90.

CLINESCHMIDT, B. V., GELLER, R. G., GOVIER, W. C. & SJOERDSMA, A. (1970). *Eur. J. Pharmac.*, **10**, 45–50.

FINCH, L. (1971). *Pharmacology*, **5**, 245–254.

HAEUSLER, G. & HAEFELY, W. (1970). *Naunyn-Schmiedebergs Arch. Pharmak.*, **266**, 18–33.

JANIS, R. A. & TRIGGLE, D. J. (1971a). *J. Pharm. Pharmac.*, **23**, 707–708.

JANIS, R. A. & TRIGGLE, D. J. (1971b). *Pharmacol. Res. Commun.* **3**, 175–182.

KALSNER, S., AYITEY-SMITH, E. & LING, G. M. (1971). *Can. J. Physiol. Pharmac.*, **49**, 566–671.

LEWIS, J. E. & MILLER, J. W. (1966). *J. Pharmac. exp. Ther.*, **154**, 46–55.

MAY, M., MORAN, J. F., KIMELBERG, A. & TRIGGLE, D. J. (1967). *Molec. Pharmac.*, **3**, 28–36.

MCGREGOR, D. D. & SMIRK, F. H. (1970). *Am. J. Physiol.*, **219**, 687–690.

MORAN, J. F., SWAMY, V. C. & TRIGGLE, D. J. (1970). *Life Sci.*, **9** (I), 1303–1315.

MORAN, J. F., TRIGGLE, C. R. & TRIGGLE, D. J. (1969). *J. Pharm. Pharmac.*, **21**, 38–46.

MORAN, J. F. & TRIGGLE, D. J. (1970). In *Fundamental Concepts in Drug Receptor Interactions*, p. 133. Editors: Danielli, J. F., Moran, J. F. & Triggle, D. J. New York: Academic Press.

NICHOLAS, T. E. (1971). *Br. J. Pharmac.*, **42**, 179–192.

OKAMOTO, K. (1969). *Intern. Rev. exp. Pathol.*, **7**, 227–270.

OKAMOTO, K. & AOKI, K. (1963). *Japan Circulation J.*, **27**, 282–293.

OKAMOTO, K., HAZAMA, F., TAKEDA, T., TABEI, R., NOSAKA, S., FUKUSHIMA, M., YAMORI, Y., MATSUMOTO, M., HAEBARA, H., ICHIJIMA, K. & SUSUKI, Y. (1966). *Ibid.*, **30**, 987–1007.

OVERBECK, H. W., SWINDALL, B. T., COWAN, D. F. & FLECK, M. C. (1971). *Circulation Res.*, **29**, 51–62.

PATIL, P. N. (1969). *J. Pharm. Pharmac.*, **21**, 628–629.

ROGERS, A. W. & MORAN, J. F. (1966). *Analyt. Biochem.*, **16**, 206–219.

ROSE, J. (1964). *Advanced Physico-Chemical Experiments*, p. 153. New York: Wiley.

SMIRK, F. H. (1970). *Circulation Res.*, **27**, *Suppl.* 11, 55–63.

SOMLYO, A. P. & SOMLYO, A. V. (1970). *Pharmac. Rev.*, **22**, 249–353.

SPECTOR, S., FLEISCH, J. H., MALING, H. M. & BRODIE, B. B. (1969). *Science*, **166**, 1300–1301.

SWAMY, V. C. & TRIGGLE, D. J. (1972). *Eur. J. Pharmac.* In the press.

TRIGGLE, D. J. (1971). *Neurotransmitter-Receptor Interactions*, Ch. IV, London and New York: Academic Press.

WOHL, A. J., HAUSLER, L. M. & ROTH, F. E. (1967). *J. Pharmac. exp. Ther.*, **158**, 531–539.

YONG, M. S. & MARKS, G. S. (1969). *Biochem. Pharmac.*, **18**, 1609–1618.