SELECTIVE ALPHA-ADRENOCEPTOR BLOCKING ACTIONS OF A NEW DERIVATIVE OF 2-HALOGENOETHYLAMINE: 6-(2-BROMOETHYL)-10,11-METHYLENEDIOXY-5,6,7,8-TETRAHYDRODIBENZ[c,e]AZOCINE

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Abstract—A new compound, 6-(2-bromoethyl)-10,11-methylenedioxy-5,6,7,8-tetrahydrodibenzo[c,e]azocine (DA-VIII-MBr) was found to have a more selective α-adrenergic blocking action than dibenamine or phenoxybenzamine. From dose-response curves for adrenaline and 5-hydroxytryptamine (5-HT) obtained in strips of rat aorta before and after incubation with each of the three blocking agents, the fractions of receptors remaining active for adrenaline and 5-HT, respectively, were estimated. After blockade with DA-VIII-MBr the receptors for adrenaline were blocked considerably, but those for 5-HT were little affected. Dibenamine blocked the receptors to adrenaline and 5-HT almost equally. The effective dose of phenoxybenzamine for adrenaline receptors was less than one hundredth that of dibenamine or DA-VIII-MBr, but specificity for these receptors was intermediate between those of dibenamine and DA-VIII-MBr. The structure of DA-VIII-MBr is an analog of apogalanthamine and its nitrogen atom bears the 2-halogenoethylamine group in part of an eight membered ring.

The analogs of apogalanthamine were first synthesized by Kobayashi and Uyeo (1) and Kotera et al. (2) who showed that some were hypotensive agents having adrenergic blocking activity. Kobayashi et al. (3) synthesized a new analog of apogalanthamine: 6-(2-bromoethyl)-10,11-methylenedioxy-5,6,7,8-tetrahydrodibenzo[c,e]azocine (DA-VIII-MBr), in which the nitrogen bears a 2-halogenoethylamine group, as they presumed it would have a potent and irreversible blocking action on α-adrenoceptors. It is of interest to note that DA-VIII-MBr has an eight membered ring in its structure, and Belleau (4, 5) showed that larger ring compounds such as benzazepine (7 membered ring) and benzazocine (8 membered ring) derivatives were potent adrenergic blocking agents, although most of 6 membered compounds exhibited no significant activity because of the rigid geometrical properties of ethyleniminium ion.

A series of 2-halogenoethylamines related to dibenamine are known to block α-adre-
neceptors (6), but they also inhibit the responses to a wide variety of other agents and exhibit various degrees of specificity and potency (7, 8, 9). For example, Furchgott (8), using strips of rabbit aorta, found that the blocking actions of dibenamine on various agonists decreased in the following order: noradrenaline and adrenaline > histamine > acetylcholine, but the actions of adrenaline and 5-hydroxytryptamine (5-HT) were blocked almost equally.

The present report deals with the selectivities of DA-VIII-MBr, compared with phenoxybenzamine and dibenamine in the irreversible blocking actions to the receptors of adrenaline and 5-HT, or to the receptors of adrenaline and histamine.

**MATERIALS AND METHODS**

Isolated strips of rat aorta were used to examine the antagonistic effects of the compounds on adrenaline and 5-HT and isolated guinea pig vas deferens to examine the antagonism to adrenaline and histamine. Male rats, weighing 300-350 g, were sacrificed by cervical fracture. Strips of aorta, an approx. width of 2-3 mm and a length of 4-5 cm, were cut spirally and mounted in organ baths having a final volume of 10 ml. The baths had a temperature of 36°C and were bubbled with air. The composition of modified Krebs-bicarbonate solution was as follows (mM): NaCl 118, KCl 4.7, CaCl$_2$ 2.2, MgCl$_2$ 3.0, Na$_2$HCO$_3$ 11.9, KH$_2$PO$_4$ 1.2 and glucose 10. Contractile responses were recorded on a smoked drum of a kymograph with an isotonic lever exerting a tension of 1.0 g and giving 20-fold amplification of responses.

Strips of rat aorta are considered adequate preparations for measuring responses to catecholamines, since they are most sensitive to noradrenaline (10) and their sensitivity is not altered by treatment with cocaine or reserpine (11). Under our experimental conditions the values of $pD_2$ were $8.39\pm 0.11$ (N = 20) for adrenaline, and $6.20\pm 0.08$ (N = 22) for 5-HT (means ± S.E., number of experiments in parentheses). The $pD_2$ values were obtained according to the method of van Rossum (12). 5-HT appears to act directly on smooth muscle, as the action was not antagonized by $10^{-7}$ M atropine or $3\times 10^{-7}$ M tetrodotoxin.

Specimens of isolated vas deferens from guinea pig, weighing 250-300 g, were also mounted in organ baths having a final volume of 10 ml. The baths had a temperature of 36°C and were bubbled with air. The composition of Tyrode solution was as follows (mM): NaCl 137, KCl 2.7, CaCl$_2$ 1.8, MgCl$_2$ 1.1, Na$_2$HPO$_4$ 0.4, Na$_2$HCO$_3$ 11.9 and glucose 10. Contractions were recorded in a similar way to those of aortic strips, but with a light tension of 0.3 g, with which the resting tension could easily be obtained and 10-fold amplification. Under the present conditions, the values of $pD_2$ were $5.51 \pm 0.03$ (N = 12) for adrenaline and $4.86 \pm 0.05$ (N = 12) for histamine (means ± S.E., number of experiments in parentheses).

For experiments on the blood pressure of pithed rats, male rats, weighing 200-300 g, were anesthetized with 0.6 ml/100 g of 25% (w/v) urethane solution in water, given i.p. Atropine sulfate, 2 mg/kg, was injected concomitantly with the urethane. Animals were pithed as described by Shipley and Tilden (13) and pressor responses to adrenaline and 5-HT were recorded from the carotid artery using a pressure transducer (Nihonkoden MP-4) and polygraph.
The agonists used were adrenaline (l-epinephrine bitartrate), 5-HT (5-hydroxytryptamine creatinine sulphate) and histamine (histamine dihydrochloride). The irreversible antagonists used were dibenamine (dibenamine hydrochloride), phenoxybenzamine (phenoxybenzamine hydrochloride) and DA-VIII-MBr** (3). Solutions of each antagonist at concentrations of $10^{-2}$ M in 0.05 N HCl in a mixture of acetone and water (1:1, v/v) were prepared on the day of use and diluted appropriately with water.

Stable responses of isolated preparations to cumulative doses of each agonist were usually obtained after equilibration for 1–2 hr with repeated washing. After treatment with a 2-halogenoethylamine, the preparations were washed repeatedly over a period of 30 min to remove unreacted amine. Measurements for cumulative dose of each agonist were repeated 2–3 times on the irreversibly blocked muscle, until stable responses to each agonist could be obtained. Contractions are expressed as percentages of the maximal response to each agonist per se.

Dose-response curves before and after irreversible blockade were plotted on semilogarithmic paper and several equipotent doses of each agonist before [A] and after [A'] blockade were determined graphically. A plot of $1/[A]$ against $1/[A']$ gave a straight line which can be described by the equation of Furchgott (14) as follows:

$$\frac{1}{[A]} = \frac{1}{q[A']} + \frac{1-q}{qK_A} \quad (i)$$

where $q$ is the fraction of the receptors not blocked by the 2-halogenoethylamine (free receptors) and $K_A$ is the dissociation constant of the agonist-receptor complex for each agonist.

RESULTS

Relative specificities of the effects of 2-halogenoethylamines on the responses of rat aortic strips to adrenaline and 5-HT

Two series of organ baths with aortic strips were set up in parallel in each experiment to measure the responses to adrenaline and 5-HT, respectively.

The recording of a typical experiment seen in Fig. 2 show that the contractile response to adrenaline was greatly inhibited, but that to 5-HT was hardly affected by pretreatment of the strips with $3 \times 10^{-8}$ M DA-VIII-MBr for 5 min. The results in Fig. 3 show that $3 \times 10^{-8}$ M dibenamine inhibited the response to 5-HT slightly more than that to adrenaline. As seen in Fig. 4, $10^{-10}$ M phenoxybenzamine inhibited the responses to both adrenaline and 5-HT, but had more effect on the response to adrenaline.

The fractions of receptors for adrenaline and 5-HT remaining after blockade were calculated from the above data by equation (i) given in the method section and the values obtained are shown in Table 1. The ratio of the fraction of remaining 5-HT receptors to that of remaining adrenaline receptors ($q_2/q_1$) was used as an index for selectivity of blockade

** Prepared in this laboratory: mp 225°C (free base). Anal. Calcd.: C$_{18}$H$_{19}$O$_2$NBr•H$_2$O: C 57.15, H 5.33, N 3.70, Found: C 57.51, H 6.58, N 3.20.
by each 2-halogenoethylamine. The ratio decreased in the order: DA-VIII-MBr > pheox- 
benzamine > dibenamine. It was also calculated from equation (i) that the $-\log K_A$ values 
of adrenaline were 7.26-7.77 by DA-VIII-MBr, 6.74-7.49 by phenoxybenzamine and 6.41- 
7.66 by dibenamine and that the $-\log K_A$ values of 5-HT were 4.60-5.47 by phenoxyben- 
zamine and 4.91-6.10 by dibenamine. No $-\log K_A$ value of 5-HT could be obtained by

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FIG. 2. Tracing of cumulative dose-responses of rat aortic strips to adrenaline (upper 
tracing: A) and 5-HT (lower tracing: B) before (a, a') and after (b, c, d and b', c', 
d') exposure to DA-VIII-MBr. Black bars indicate successive exposures to 
$3 \times 10^{-8} \text{M DA-VIII-MBr}$ for 5 min. In the upper tracing, note that the response 
to 5-HT (e) remains even after the complete blockade to adrenaline, while that to 
adrenaline (e') is blocked in the lower tracing.

FIG. 3. Tracing of cumulative dose-response of rat aortic strips to adrenaline (upper 
tracing: A) and 5-HT (lower tracing: B) before (a, a') and after (b, c and b', c') 
exposure to dibenamine. Black bars indicate successive exposures to $3 \times 10^{-8} \text{M dibenamine}$ for 5 min.
Fig. 4. Tracing of cumulative dose-responses of rat aortic strips to adrenaline (upper tracing: A) and 5-HT (lower tracing: B) before (a, a') and after (b, c, d and b', c', d') exposure to phenoxybenzamine. Black bars indicate successive exposure to $10^{-10}$ M phenoxybenzamine for 5 min.

Table 1. Fractions of remaining receptors of adrenaline and 5-HT after irreversible blockade of rat aortic strips

| Exposure                       | Remaining receptors | Ratio | p-Value* |
|--------------------------------|---------------------|-------|----------|
|                                | Adrenaline ($q_1$)  | 5-HT  ($q_2$) | $(q_2/q_1)$ |          |
| A) After $3 \times 10^{-8}$ M DA-VIII-MBr for 5 min | 1st 0.199±0.030 (6) | 0.702±0.032 (6) | 3.53 | < .01 |
|                                | 2nd 0.066±0.010 (4) | 0.541±0.034 (4) | 8.20 | < .01 |
| B) After $10^{-10}$ M phenoxybenzamine for 5 min | 1st 0.207±0.046 (6) | 0.396±0.099 (6) | 1.90 | < .05 |
|                                | 2nd 0.047±0.011 (4) | 0.138±0.025 (4) | 2.96 | < .05 |
| C) After $3 \times 10^{-8}$ M dibenamine for 5 min | 1st 0.179±0.025 (5) | 0.127±0.028 (5) | 0.71 | < .05 |
|                                | 2nd 0.031±0.011 (4) | 0.051±0.033 (4) | 1.65 | N.S.** |

Means±S.E. Number of experiments in parentheses.
* Level of significance of difference between remaining receptors of adrenaline and 5-HT on statistical analysis by the paired two tailed t-test.
** N.S.: Not significant.

DA-VIII-MBr, because at the concentration used the maximum contraction induced by 5-HT was not inhibited. The $-\log K_a$ values for each agonist were varied to the extent that differences among the values by the three antagonists were not statistically significant.

Relative selective effects of 2-halogenoethylamines on the responses of guinea pig vas deferens to adrenaline and histamine

Preparations of guinea pig vas deferens were used for measurement of responses to adrenaline and histamine. Concentrations of antagonists used here, being higher than
TABLE 2. Fractions of remaining receptors of adrenaline and histamine after irreversible blockade of guinea-pig vas deferens

|          | Adrenaline ($q_0$) | Histamine ($q_0$) | Ratio ($q_0/q_0$) |
|----------|-------------------|-------------------|------------------|
| A) After 10^{-7} M DA-VIII-MBr for 30 min | 0.044±0.010 (7) | 0.767±0.058 (7) | 17.6             |
| B) After 10^{-9} M phenoxybenzamine for 30 min | 0.048±0.001 (4) | 0.720±0.092 (4) | 15.0             |
| C) After 3×10^{-7} M dibenamine for 30 min | 0.090±0.013 (5) | 0.598±0.049 (5) | 6.6              |

Means±S.E. Number of experiments in parentheses.

those on the aortic strips, were 10^{-7} M of DA-VIII-MBr, 3×10^{-7} M of dibenamine and 10^{-9} M of phenoxybenzamine and these preparations were exposed for a longer period of 30 min. All these antagonists blocked the response to adrenaline more than that to histamine, but their selective effects decreased in the order of DA-VIII-MBr>phenoxybenzamine >dibenamine. From Table 2 it is evident that the difference in selectivity of each antagonist was greater in the histamine than in the 5-HT-experiments.

**Effects of 2-halogenoethylamines on responses of the blood pressure of pithed rats to adrenaline and 5-HT**

The mean blood pressure of pithed rats is usually between 20 and 40 mmHg. First the pressor responses with graded doses of 0.5–10 μg/kg of adrenaline and 50–300 μg/kg of 5-HT were usually recorded. A suitable dose of a 2-halogenoethylamine was then injected...
into the femoral vein and 30 min later the responses to graded doses of adrenaline and 5-HT were again estimated. The results in Fig. 5 show that doses of 0.2–0.4 mg/kg of DA-VIII-MBr potentiated rather than inhibited the response to 5-HT, however, the response to adrenaline was inhibited. Doses of 0.1–0.2 mg/kg of dibenamine blocked the response to 5-HT more than that to adrenaline, whereas 0.1–0.2 mg/kg of phenoxybenzamine inhibited the response to adrenaline much more than that to 5-HT. The selectivities of these antagonists in inhibition of the effects of adrenaline and 5-HT were similar to those on the rat aortic strip.

DISCUSSION

2-Halogenoethylamines related to dibenamine were once considered to block specifically α-adrenoceptors. There is now evidence to the contrary. For example, N-ethyl-N-1’-naphthylethyl-2-bromoethylamine (SY-28) inhibits the action of histamine more than that of adrenaline (15), and more recently, benzilylcholine mustard was found to be a potent antagonist of the muscarinic action of acetylcholine (16). In the present work, the effects of 2-halogenoethylamines on the actions of adrenaline and 5-HT on isolated rat aortic strips and on the blood pressure of pithed rats were examined and DA-VIII-MBr was found to inhibit the action of adrenaline more specifically than dibenamine or phenoxybenzamine.

Since the discovery of the action of dibenamine, the effects of many more less structurally related compounds have been investigated. However, various difficulties have been encountered in elucidating the relation of structure to activity of the compounds at a molecular level. The pharmacologically active species were found to be the ethyleniminium ions generated from the parent 2-halogenoethylamines, as demonstrated by many workers (17, 18, 19, 20). In the case of any parent compound, the concentration of the active species at any time depends on both the rate of cyclization to the ethyleniminium ions and the rate of hydrolysis of the latter ion, and therefore is difficult to measure when an antagonist is used. To represent antagonistic activities on agonists in terms of the concentration of 2-halogenoethylamine added is not feasible since these activities change considerably with factors such as the period after preparation of the solutions and the temperature.

In our experiments, two series of organ baths with isolated smooth muscles were set up in parallel for assay of the responses to two agonists. Thus, although the actual concentrations of active species of the 2-halogenoethylamines are unknown, the relative antagonistic activities against two agonists could be determined under similar conditions. The fractions of remaining receptors for adrenaline and 5-HT were estimated from the dose-response curves before and after irreversible blockade by each antagonist. DA-VIII-MBr was found to be the most selective inhibitor of adrenaline, though its inhibitory activity was not greater than that of phenoxybenzamine as shown in Table 1, when estimated by means of the concentrations of antagonists prepared. After blockade by two successive 5 min exposures to $3 \times 10^{-4}$ M DA-VIII-MBr, 54.1% of the receptors of 5-HT and only 6.6% of the receptors of adrenaline remained unblocked. The effective dose of phenoxybenzamine was less than one hundredth of that of DA-VIII-MBr or dibenamine, but the
specificity was intermediate between those of the latter compounds. Dibenamine was about as powerful an inhibitor to adrenaline as DA-VIII-MBr, but it inhibited also the response to 5-HT equally. The specific antagonistic action of adrenaline after DA-VIII-MBr was also demonstrated by studying the effects on responses of guinea pig vas deferens to adrenaline and histamine, and of the blood pressure of pithed rats to adrenaline and 5-HT.

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