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Competitive blockade of adrenergic α-receptors and histamine receptors by thymoxamine

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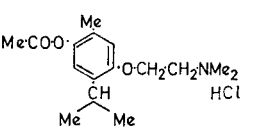
The adrenergic α-receptor blocking activity of 4-(2-dimethylaminoethoxy)-5-isopropyl-2-methylphenyl acetate, thymoxamine, was quantitatively investigated on the vas deferens of the guinea-pig and on arterial strips from guinea-pigs, rabbits, cats and dogs. The blockade fulfilled the established criteria for competitive antagonism of noradrenaline. On these various tissues the pA_2 was about 7·0; thymoxamine was more potent than piperoxan and less potent than dihydroergotamine. There was no evidence of β-receptor or 5-hydroxytryptamine receptor blocking activity. Against histamine as the agonist on the guinea-pig ileum, thymoxamine was a competitive antagonist with a pA_2 of about 6·5.

THE benzodioxans and phenoxyalkylamines have been extensively studied as agents blocking adrenergic α-receptors. From the thymyl ether of an alkylamine the first useful antihistamine drugs were developed but the α-blocking activity of thymoxyalkylamines does not seem to have been the subject of quantitative investigation of mechanism of blockade (see Barlow, 1964).

Greef & Schümann (1953) described 4-(2-dimethylaminoethoxy)-5-isopropyl-2-methylphenyl acetate (I)* as a sympathicolytic and histaminolytic agent. The compound has since been used clinically for a range of vascular disorders.



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This paper reports the results of quantitative experiments on isolated tissues which suggest that thymoxamine is a competitive antagonist at adrenergic α-receptors and at histamine receptors.

Experimental

METHODS

The isolated tissues were suspended in jacketed organ baths containing 20 ml of Krebs solution bubbled with 95% oxygen and 5% carbon dioxide. Contractions were recorded on smoked paper with isotonic frontal writing levers.

Guinea-pig vas deferens (Leach, 1956). Each vas deferens was removed without the hypogastric nerve and set up in Krebs solution at 32°; the load on the tissue was 0·5 g; the magnification was 4 times. Agonist drugs were added to the bath for 1 min in each 5 min period.

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* Proposed name Thymoxamine ("Opilon" Wm. R. Warner and Co. Ltd.).

Arterial strips (Furchgott & Bhadrakom, 1953). Spirally-cut strips were prepared from the thoracic or abdominal aorta of rabbits, guinea-pigs or cats and from the carotid artery of dogs. The Krebs solution was maintained at 37° for rabbit, cat and dog strips and at 32° for strips from guinea-pigs; the load was 0·25 g; the magnification was 10 times for rabbit, cat and dog and 20 times for guinea-pig. Agonist drugs were added to the bath for 3 min in each 15 min period (rabbits, guinea-pigs, dogs) or 2 min in each 10 min period (cats).

Guinea-pig ileum. Middle ileum in 3 cm lengths was used at 37° loaded at 0·5 g with a magnification of 4 times. The agonist, histamine, was added for a contact time of 1 min during each 4 min period.

Rat fundus (Vane, 1957). Longitudinal strips were kept at 37°, loaded at 0·5 g, with a magnification of 10 times. Dose response curves to 5-hydroxytryptamine (5-HT) were obtained in the absence of and in the presence of thymoxamine.

Guinea-pig auricles (Giotti, 1954). The isolated auricles were kept at 37° and spontaneous beats recorded by a spring-loaded side-writing lever on smoked paper. Adrenaline was added to the bath for a 1 min contact time. Responses to adrenaline were recorded in the absence of antagonist and after 2 or 15 min exposure to antagonist.

DRUGS

Drugs used were: acetylcholine chloride, (−)-adrenaline bitartrate, angiotensin II (Ciba), dihydroergotamine methane sulphonate, histamine acid phosphate, 5-hydroxytryptamine creatinine sulphate, (−)-noradrenaline bitartrate, piperoxan hydrochloride, propranolol hydrochloride, thymoxamine hydrochloride.

Concentrations are expressed as molar final bath concentration or in terms of the base.

Results

VAS DEFERENS

The threshold dose of noradrenaline was 2 or 4 $\times$ 10^{-6} M and the response increased with twofold increases in concentration up to maximal responses at 1·28 or 5·12 $\times$ 10^{-4} M. Initial dose response curves were repeated (usually three times) until the response of the vas was constant. Dose response curves to noradrenaline were repeated in the presence of a range of concentrations of thymoxamine which was added to the bath 2 min before each dose of noradrenaline. Concentrations of thymoxamine from 1 $\times$ 10^{-8} to 1·8 $\times$ 10^{-6} M moved the log dose response curves for noradrenaline to the right of the curves for noradrenaline alone. The curves in the presence of antagonist were parallel to the curves in the absence of antagonist. The heights of the maximal responses were not reduced by thymoxamine.

The horizontal distance between the curves obtained in the absence of and presence of thymoxamine was measured at 10% intervals from 10 to 80% of maximum response (Fig. 1). This distance was expressed as a dose ratio (Gaddum, Hameed, Hathway & Stephens, 1955) and the means of these dose ratios for four different concentrations of thymoxamine were plotted

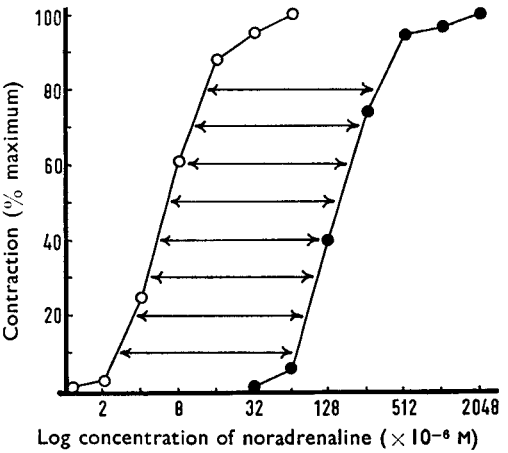

FIG. 1. Log dose response curves from a single experiment with a guinea-pig isolated vas deferens preparation. The curve for noradrenaline alone (○——○) and the curve for noradrenaline in the presence of $1{\cdot}8 \times 10^{-6}$M thymoxamine (●——●) for which the antagonist was added to the bath 2 min before each dose of noradrenaline. The arrows indicate the horizontal distances measured at each 10% of maximum interval (from 10% of maximum to 80% of maximum inclusive). These distances were converted to dose ratios for noradrenaline.

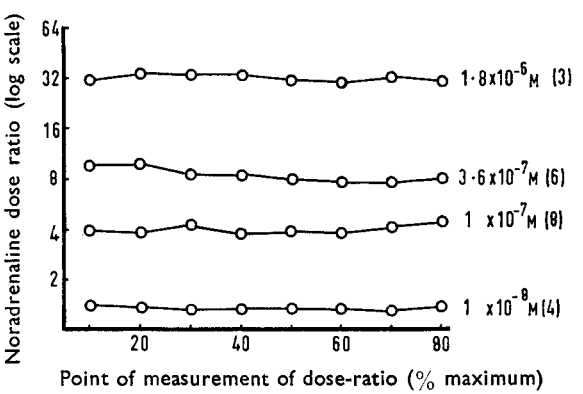

FIG. 2. The means of the noradrenaline dose-ratios determined on the guinea-pig vas deferens (measured as shown in Fig. 1) for four different concentrations of thymoxamine. The concentration of thymoxamine used is shown against each curve. The numbers in brackets indicate the number of vasa used to determine the means.

over the 10 to 80% range of response (Fig. 2). The mean dose ratios for each concentration of thymoxamine seem to lie on straight lines which are parallel to the abscissa.

The relation between dose ratio and antagonist concentration was analysed by the method of Arunlakshana & Schild (1959). The logarithm of (x — 1), where x equals the noradrenaline dose ratio, was plotted against the negative logarithm of B, where B is the molar concentration of thymoxamine (Fig. 3). The points for the four concentrations of

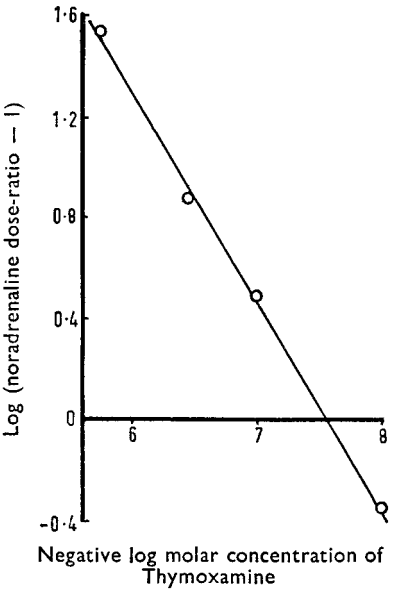

FIG. 3. Results from guinea-pig isolated vas deferens plotted by the method of Arunlakshana & Schild (1959). A calculated regression line is fitted to the four points. Each point is a mean, derived from the data shown in Fig. 2, for each concentration of thymoxamine. The line intercepts the abscissa at the pA_2 value of 7·57. Where log (x-1) equals 0·95 a perpendicular dropped from the regression line to the abscissa gives the pA_{10} value of 6·42.

thymoxamine lay on a straight line. The calculated regression line intercepted the abscissa at the pA_2 value 7·57. When measured directly in four experiments, by the method of Schild (1947), the pA_2 was 7·56. The pA_2–pA_{10} value determined from Fig. 3 was 1·15.

From the formula $\log (x - 1)/B = \log K_2$, values of $\log K_2$ were calculated for each dose ratio at each of the four molar concentrations of thymoxamine. The mean of these four values for $\log K_2$ was 7·44, which was close to the pA_2 value.

In 5 experiments the course of the reversibility of the blockade was estimated. With the thymoxamine added before the noradrenaline the rate of return of pre-antagonist sensitivity to noradrenaline ranged from 6 washes in 8 min for $1{\cdot}25 \times 10^{-8}$ M thymoxamine, to 45 washes in 75 min for 1×10^{-5} M thymoxamine. After 20 min exposure to 1×10^{-7} M thymoxamine 15 washes in 30 min restored sensitivity.

The pA_2 for dihydroergotamine was also measured, with the antagonist continuously present in the Krebs solution for a period of 30 min. Under these conditions the pA_2 for dihydroergotamine against noradrenaline was 8·25.

ARTERIAL STRIPS

Rabbit aorta. Measurements of pA_2 for thymoxamine against noradrenaline contractions were made after 10, 25 and 40 min contact

with thymoxamine on four rabbits (Fig. 4). The mean pA_2 values were 6·80, 6·88 and 6·90 respectively. pA_2 values for piperoxan similarly determined were 6·28, 6·39 and 6·39.

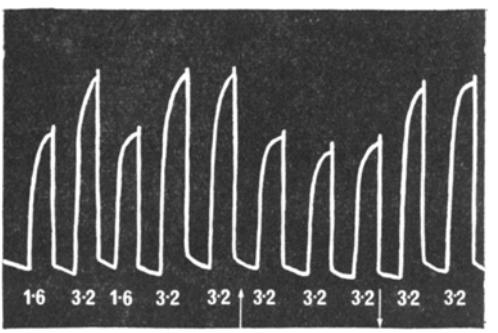

FIG. 4. Contractions of a rabbit aortic strip to noradrenaline. The concentration of noradrenaline used is shown by the figure beneath each contraction ($\times 10^{-7}$ molar). Contact time 3 min; 15 min cycle; three changes of bath fluid after each contraction. At the first arrow thymoxamine was added to the Krebs, to a final concentration of 8×10^{-8} M 7 min before the next contraction to noradrenaline. This concentration of thymoxamine was present in the Krebs until washed out at the second arrow, and it reduced the size of the response to the double dose of noradrenaline (3·2) to about that of the single dose (1·6). The rapidity of onset of the blockade and its ease of reversal by washing can be judged from this record.

Using aortic strips from three rabbits the effect of thymoxamine on contractions produced by angiotensin was observed. Concentrations from 1×10^{-7} to $2{\cdot}5 \times 10^{-5}$ M did not reduce the response of the aorta to angiotensin.

Guinea-pig aorta. Concentrations of 1 or 2×10^{-6} M thymoxamine were used on aortic strips from three guinea-pigs; in each experiment there was a parallel shift of the noradrenaline dose response curve to the right.

The pA_2 was measured on aortic strips from five guinea-pigs; the mean value was 7·20 for a 5 min antagonist contact period.

Cat aorta. Dose response curves to noradrenaline were made on aortic strips from three cats in the absence of and in the presence of thymoxamine concentrations from 1×10^{-7} to $6{\cdot}4 \times 10^{-6}$ M. The sensitivity of the strips and the slopes of the control dose response curves varied during an experiment. Thymoxamine moved the dose response curve to the right but the shift was not always parallel, the dose ratios being smaller nearer the maximum. The height of the maximum was not reduced. Estimates of the pA_2 from the dose ratio at the 50% of maximum point gave a mean value of 6·10.

Dog carotid. On carotid strips taken from four dogs, the pA_2 for thymoxamine against noradrenaline was measured by the method of Schild (1947). The mean value was 6·99 for a 13 min antagonist contact and 7·01 for 28 min contact.

GUINEA-PIG ILEUM

On sections of ileum from five guinea-pigs, dose response curves to histamine were made in the absence of and in the presence of thymoxamine in concentrations from 1×10^{-7} to $2{\cdot}56 \times 10^{-5}$ M. The curves in the

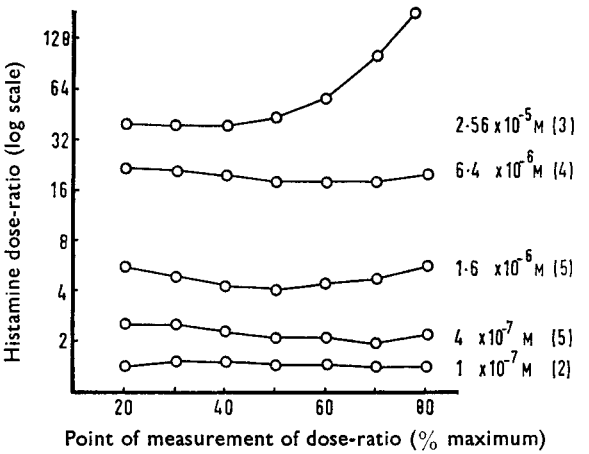

FIG. 5. The means of the histamine dose-ratios determined on the guinea-pig ileum for five different concentrations of thymoxamine. The concentration of thymoxamine used is shown against each curve. The numbers in brackets indicate the number of guinea-pigs used to determine the means.

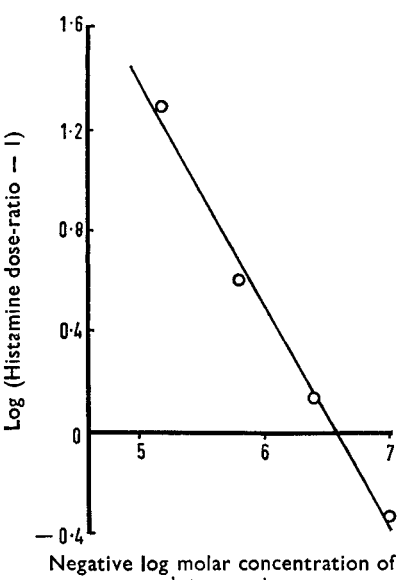

FIG. 6. Results from guinea-pig isolated ileum plotted by the method of Arunlakshana & Schild (1959). A calculated regression line is fitted to the four points. Each point is a mean, derived from the data shown in Fig. 5, for each concentration of thymoxamine. The line intercepts the abscissa at the pA_2 value of 6·57. Where log (x-1) equals 0·95 the pA_{10} on the abscissa equals 5·50.

presence of antagonist were to the right of those in the absence of antagonist, all the curves were parallel except for the highest concentration of thymoxamine, $2{\cdot}56 \times 10^{-5}$ M (Fig. 5). Plots of log (x — 1) against B, where x equalled the histamine dose ratio and B the molar concentration of thymoxamine, were made (Fig. 6). The calculated regression line

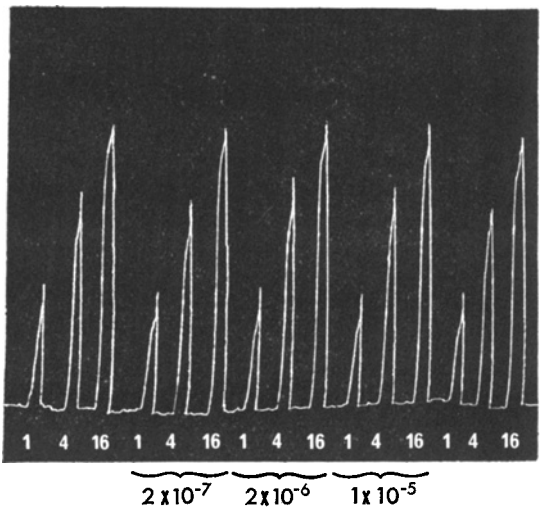

FIG. 7. Contractions of a rat isolated stomach fundus strip to 5-hydroxytryptamine. The concentration of 5-HT used is shown by the figure beneath each contraction ($\times 10^{-9}$ g/ml). Contact time one min; 5 min cycle; 5 changes of bath fluid after each contraction. The first three responses were in the absence of thymoxamine, the following three groups of three responses were in the presence of the concentrations of thymoxamine shown beneath the tracing, added 2 min before each 5-HT dose. The last group of three responses was again in the absence of thymoxamine.

intercepted the abscissa at the pA_2 value of 6·57. The pA_{10} from the curve was 5·5, so that pA_2–pA_{10} was 1·07. Log K_2 values were calculated for each of the four concentrations of thymoxamine used. The mean of these four log K_2 values was 6·52, which was close to the pA_2.

Wash recovery was measured in three guinea-pigs. Time of recovery to original sensitivity with repeated washing varied from 32 min with 4×10^{-7} M thymoxamine to 100 min with $6{\cdot}4 \times 10^{-6}$ M.

RAT FUNDUS

On fundus strips from three rats, concentrations of thymoxamine up to 1×10^{-5} M, added to the bath 2 min before the addition of the 5-HT agonist, did not alter the position of the 5-HT dose response curves (Fig. 7).

GUINEA-PIG AURICLES

On isolated auricles from three guinea-pigs concentrations of thymoxamine up to 1×10^{-5} M acting for 15 min did not change the effect of adrenaline (Fig. 8) whereas propranolol, 1×10^{-7} M, completely abolished the response to adrenaline.

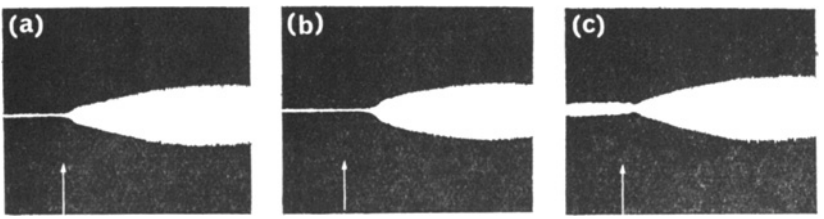

FIG. 8. Spontaneous contractions of guinea-pig isolated auricles. In panel (a) the response to a bath concentration of 8×10^{-8} g/ml adrenaline, acting for 1 min. Between (a) and (b) thymoxamine was added to a concentration of 1×10^{-6} molar 2 min before repeating the dose of adrenaline. Between (b) and (c) the auricles were exposed to a concentration of 1×10^{-5}M thymoxamine for 15 min before repeating the adrenaline dose. The adrenaline doses were added at the arrows.

TABLE 1. pA_2 VALUES

Tissue	Antagonist	pA_2	Remarks
Adrenergic α-receptor (agonist: noradrenaline)			
Guinea-pig vas deferens	Thymoxamine	7·57	Calculated from Fig. 3
	Dihydroergotamine	8·25	Antagonist contact time 30 min
	Piperoxan	6·47	Calculated from results of Leach (1956). Antagonist added 5 min before each agonist dose
Rabbit aortic strip ..	Thymoxamine	6·80	Antagonist contact time: 10 min
		6·88	Antagonist contact time: 25 min
		6·90	Antagonist contact time: 40 min
	Piperoxan	6·28	Antagonist contact time: 10 min
		6·39	Antagonist contact time: 25 min
		6·39	Antagonist contact time: 40 min
Guinea-pig aortic strip	Thymoxamine	7·20	Antagonist contact time: 5 min
Dog carotid strip ..	Thymoxamine	6·99	Antagonist contact time: 13 min
		7·01	Antagonist contact time: 28 min
Cat aortic strip ..	Thymoxamine	6·10	Calculated from dose response curves
Histamine receptor (agonist: histamine)			
Guinea-pig ileum ..	Thymoxamine	6·57	Calculated from Fig. 6
	Mepyramine	9·3	Arunlakshana & Schild (1959). Antagonist 14 min before agonist

Discussion

Some qualitative aspects of the pharmacology of thymoxamine were reported by Greef & Schümann (1953). They showed that thymoxamine abolished the response of the rabbit uterus and the guinea-pig seminal vesicle to adrenaline. Thymoxamine reduced but did not abolish adrenaline inhibition of the rabbit small intestine. Intravenous injections of thymoxamine lowered the blood pressure of the rabbit, cat or dog and reduced or blocked the hypertensive effect of injections of adrenaline or noradrenaline. Adrenaline reversal was seen in the dog but not in the cat or rabbit. The rise in blood pressure produced in the rabbit by

stimulation of the central end of the sciatic nerve, or in the cat by the carotid occlusion reflex, was reduced by thymoxamine. Contraction of the cat nictitating membrane by adrenaline was reduced by thymoxamine. On the guinea-pig ileum, thymoxamine slightly reduced the response to acetylcholine, blocked the response to histamine and did not reduce the response to barium chloride. Greef & Schümann concluded that thymoxamine had what they called sympathicolytic and histaminolytic properties but drew no conclusions about the mode of action of the drug.

The experiments now reported were made in an attempt to characterise the nature of the blockade of adrenergic or histamine receptors produced by thymoxamine. A second object was to measure the potency of thymoxamine.

In differentiating between competitive and non-competitive antagonism, a number of criteria for competitive antagonism have been proposed in the past (Gaddum, 1937, 1957; Schild, 1947, 1957; Furchgott, 1955; Arunlakshana & Schild, 1959). As an antagonist of noradrenaline at α-receptors on the vas deferens and on vascular smooth muscle and as an antagonist of histamine on the guinea-pig ileum, thymoxamine was found to fulfil these requirements of competitive antagonism.

(i) For both noradrenaline and histamine the log dose response curves in the presence of various concentrations of thymoxamine were to the right of and parallel to those in the absence of the antagonist. The antagonism was surmountable, maximal responses being of the same height in the presence of antagonist as in its absence.

(ii) The quantitative criteria were found to be satisfied. Over a 180-fold dose range for thymoxamine on the vas deferens there was a linear relation between dose-ratio of noradrenaline minus one, and concentration of antagonist. The pA_2 taken from this relation was 7·57 and was the same as that found by a separate direct determination (7·56). The value of pA_2–pA_{10} at 1·15 was in close agreement with the theoretical value of 0·95 for competitive antagonism. Furthermore, the mean of the four calculated log K_2 values at 7·44 was near to the pA_2 value of 7·57 which is indicative of the competitive nature of the antagonism.

With histamine as the agonist on the guinea-pig ileum there was a parallel shift of the dose response curves to the right over a 64-fold range of thymoxamine concentration. From the regression line fitted to the plot of dose ratio minus one against antagonist concentration, the pA_2–pA_{10} value of 1·07 was near to the theoretically required value of 0·95 for competitive antagonsim and the mean log K_2 value of 6·52 was close to the pA_2 of 6·57.

(iii) The antagonism of noradrenaline at α-receptors or of histamine at histamine receptors was completely reversible by repeated washing of the tissue with fresh Krebs solution. The amount of washing and the period of time needed to restore the original sensitivity to the agonist depended on the antagonist concentration used.

(iv) For the α-receptor, the pA_2 values measured on tissues from different species and different tissues from the same species were, except for the cat aorta, closely similar (Table 1). The pA_2 value in the cat

was measured from dose response curves which varied more than those from the other species. The cat aortic strips were thicker than aortic strips from rabbits or guinea-pigs or the carotid strips from dogs; this is the most noticeable difference and may account for the differences in pA_2 values.

The potency of thymoxamine may be judged from Table 1. As an antagonist at α-receptors thymoxamine is more potent than piperoxan and less potent than dihydroergotamine. At the histamine receptor thymoxamine is a relatively weak antagonist when compared with an established antihistamine drug such as mepyramine.

The relative specificity of thymoxamine for α-sympathetic receptors was indicated by its lack of effect on the response of the guinea-pig auricles to adrenaline, on which tissue thymoxamine appeared to have no β-receptor blocking activity. The specificity was further suggested by the lack of blocking action against contractions induced by angiotensin on the rabbit aorta. Again, the inability of thymoxamine to reduce the response of the rat fundal strip to 5-HT was evidence for the lack of blocking action at 5-HT receptors.

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