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Antagonism of the response of human isolated arteries to noradrenaline

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1. Spirally-cut strips from human visceral arteries isolated *in vitro* were contracted by noradrenaline and this contraction was antagonized by the α-receptor blocking agent, thymoxamine.

2. The cumulative log dose-response curves to noradrenaline in the presence of thymoxamine were moved to the right of and parallel to the curve for noradrenaline alone. The blockade was surmountable and reversible.

3. The quantitative criteria for simple competitive antagonism were satisfied. When log $(x-1)$ was plotted against pA_x the points fitted a straight line with a slope $(-n)$ of 0.91. The pA_2 for thymoxamine was 7.54 and the mean value for log K_2 was 7.82. These values are in approximate agreement with those previously reported for animal isolated arterial strips.

In recent years there have been several reports of the use of human tissues isolated *in vitro* for pharmacological experiments, but human blood vessels seem to have been little used, despite their apparent ready availability. Cutchin, McGaughey, Scoggin, Harbert & Thornton (1964) investigated the responses to noradrenaline of segments of human uterine artery removed at operation and de la Lande, Cannell & Waterson (1966) measured vasoconstrictor responses to noradrenaline of perfused digital arteries removed soon after death. In addition to information about the response of human arteries to noradrenaline, it would also be of interest to have, for both theoretical and therapeutic reasons, information on the mode of action of α-receptor blocking agents on human arteries. The adrenergic blocking agent thymoxamine, 4-(2-dimethylaminoethoxy)-5-isopropyl-2-methylphenyl acetate, has been in use clinically for vasospastic disorders, and its mode of action on animal isolated arteries was investigated by Birmingham & Szolcsányi (1965). We have now made a quantitative analysis of the mode of action and of the potency of thymoxamine on human isolated visceral arteries.

Methods

The arteries were removed during intra-abdominal operations from patients who were not known to have any arterial or other vascular disease. Anaesthesia was induced with thiopentone and maintained with nitrous oxide and oxygen and pethi-

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dine ; the muscle relaxants used were suxamethonium and tubocurarine ; premedication was omnopon 20 mg and scopolamine 0.4 mg. Immediately after removal, the artery (about 2 or 3 cm in length) was placed in a vacuum flask containing approximately 200 ml. of Krebs solution at 4° C, which had been gassed with a mixture of 95% oxygen and 5% carbon dioxide and transported to the laboratory. About 1 hr elapsed between the excision of the artery and the beginning of the *in vitro* experiment, for which purpose the artery was cut spirally to make a strip with the circular muscle fibres running along the length of the strip (Furchgott & Badrakom, 1953). It was then usually possible to make two preparations (each about 2 mm wide) by dividing the strip by a further longitudinal cut. Each arterial strip was then suspended in a jacketed organ bath containing 100 ml. of Krebs solution containing 6×10^{-4}M ascorbic acid at 37° C bubbled with 95% oxygen and 5% carbon dioxide. Each strip was attached at its upper end to an isotonic frontal writing lever of 10 times magnification ; the tissue was loaded with 1.5 g and contractions were recorded on smoked paper. After allowing the artery to equilibrate with the Krebs solution for 2 hr, cumulative log dose-response curves to noradrenaline were recorded. Noradrenaline was added to reach a final bath concentration of 1×10^{-7}M, allowed to act for 3 min and then the concentration of noradrenaline was doubled every 3 min until further increase in concentration produced no increase in contraction. When the maximal response had thus been achieved, the kymograph was stopped and the drug washed out by repeated replacement of the bath fluid with fresh Krebs solution until the artery had relaxed to its original length. Next, the normal Krebs solution was replaced by Krebs solution containing the first concentration of the antagonist drug, thymoxamine 1×10^{-8}M, and 2 min later the cumulative log dose-response curve to noradrenaline was re-established in the presence of the antagonist. When the maximal response to noradrenaline had again been reached, the artery was washed with Krebs solution (still containing 10^{-8}M thymoxamine) until it had relaxed to the pre-noradrenaline baseline. The Krebs solution was then changed to one containing 10^{-7}M thymoxamine and the response to noradrenaline was again recorded. This procedure was repeated until cumulative log dose-response curves to noradrenaline had been recorded in the presence of 10^{-6}M and finally, 10^{-5}M thymoxamine.

On one occasion, the pA_2 for thymoxamine against noradrenaline was measured by the direct method of Schild (1947) by ascertaining that concentration of thymoxamine needed to reduce the height of contraction of an arterial strip induced by a double dose of noradrenaline to that of a single dose of noradrenaline.

The drugs used were (−)-noradrenaline bitartrate and thymoxamine hydrochloride.

Results

Strips from a gastro-epiploic, a splenic and a left ovarian artery were used for the mean results shown as log dose-response curves in Fig. 1. The threshold concentration of noradrenaline was about 1×10^{-7}M and with each succeeding increase in bath concentration of noradrenaline there was an increase in the height of the contraction until a maximal response was reached at about 4×10^{-4}M. The presence of 1×10^{-8}M thymoxamine rendered these arteries slightly more sensitive to noradrenaline, as shown by the small shift of the log dose-response curve to the left of that for noradrenaline alone. When the concentration of thymoxamine was

increased to 1×10^{-7}M, the arteries became much less sensitive to noradrenaline, the log dose-response curve was now moved to the right of and parallel to that for noradrenaline in absence of the antagonist. In the same way, two more ten-fold increases in antagonist concentration moved the noradrenaline log dose-response curves further to the right of the curve for the agonist alone. At all concentrations of thymoxamine the antagonism was surmountable by noradrenaline; it was possible to re-establish the maximal response to noradrenaline. The antagonism was reversible by prolonged washing of the tissue in normal Krebs solution.

For the three concentrations of thymoxamine, 10^{-7}, 10^{-6} and 10^{-5}M, which caused a right-ward shift in the noradrenaline log dose-response curve, noradrenaline dose-ratios were calculated from measurements of the horizontal distances between the curves obtained in the absence of, and in the presence of, thymoxamine. Measurements were made at 10% intervals from 10 to 80% of maximum response and the mean of these eight measurements was calculated for each concentration of thymoxamine.

The relation between noradrenaline dose ratio and thymoxamine concentration was analysed by the method of Arunlakshana & Schild (1959). The logarithm of the noradrenaline dose ratio minus 1 was plotted against the negative logarithm of the molar concentration of thymoxamine (Fig. 2). The points for the three concentrations of thymoxamine lay on a straight line and the calculated regression line had a slope $(-n)$ of 0.91 and intercepted the abscissa at the pA_2 value 7.54.

From the formula $\log (x-1)/B = \log K_2$ (Arunlakshana & Schild, 1959), where x is the noradrenaline dose ratio and B is the molar concentration of thymoxamine, values of $\log K_2$ were calculated for each of the three concentrations of thymoxamine. The mean of these three values for $\log K_2$ was 7.82, which was close to the pA_2 value.

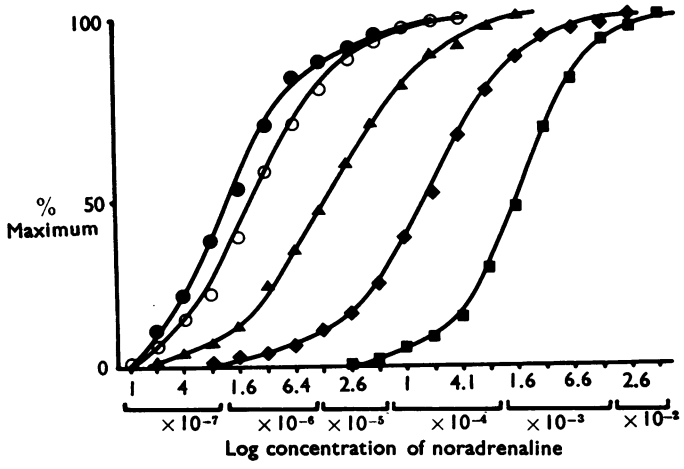

FIG. 1. Cumulative noradrenaline log dose-response curves from the mean responses of a human splenic, left ovarian and gastro-epiploic arterial strip isolated in Krebs solution at 37° C. Ordinate: contraction height expressed as a percentage of the maximum response. Abscissa: molar bath concentration of noradrenaline on a logarithmic scale. Response to noradrenaline alone —○—; in the presence of thymoxamine —●— 10^{-8}M, —▲— 10^{-7}M, —◆— 10^{-6}M and —■— 10^{-5}M.

On one occasion, a right gastric arterial strip was used to measure the pA_2 for thymoxamine by the direct method of Schild (1947). By this method the pA_2 was 7.37.

The responses of two diseased arteries were also examined. A skeletal muscle artery from a diabetic patient seemed to respond normally to noradrenaline and the pA_2 for thymoxamine (by the method of Arunlakshana & Schild, 1959) was 7.30. A right external iliac artery, which had in the past been unblocked surgically (" disobliterated ") and which on cutting with scissors appeared to be very sclerotic, did not contract in the presence of noradrenaline.

Discussion

The experiments reported here show that human visceral arteries, isolated *in vitro* as strips of circular muscle prepared by the method of Furchgott & Badrakom (1953), are contracted by noradrenaline. The response to noradrenaline is antagonized by the α-receptor blocking agent thymoxamine and a quantitative analysis of the characteristics of the blockade has shown the antagonism to be competitive. The following criteria for simple competitive antagonism were fulfilled by the blockade of noradrenaline by thymoxamine.

(i) The log dose-response curves for noradrenaline in the presence of three concentrations of thymoxamine were moved progressively to the right of and parallel to the curve for noradrenaline alone. The antagonism was surmountable, maximal responses being the same height in the presence of the antagonist as in its absence.

(ii) Over a one hundred-fold dose range for thymoxamine there was a linear relation between the log dose-ratio minus one of agonist and log concentration of

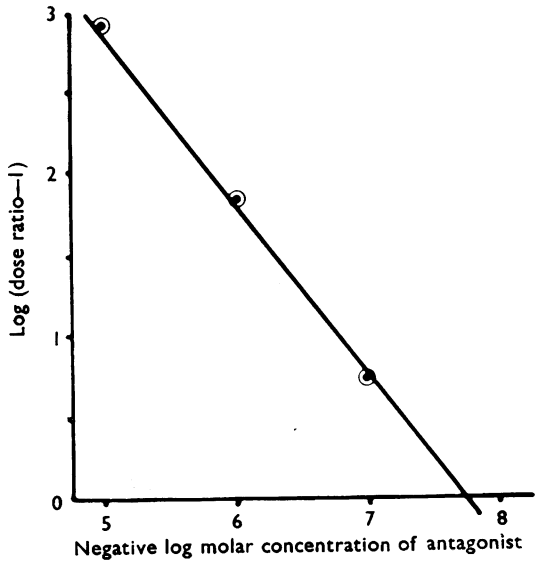

FIG. 2. Results from Fig. 1 for the three highest concentrations of thymoxamine plotted by the method of Arunlakshana & Schild (1959). Ordinate: logarithm of (noradrenaline dose ratio -1). Abscissa: negative logarithm of the molar concentration of thymoxamine. A calculated regression line fitted to the three points has a slope $(-n)$ of 0.91 and intercepts the abscissa at the pA_2 value of 7.54.

antagonist. The slope ($-n$) of the regression line fitted to the three points was 0.91 and sufficiently close to the theoretically required value of 1 to support a hypothesis of simple competitive antagonism between noradrenaline and thymoxamine. The pA_2 value based on the relation was 7.54 and similar to that obtained by a separate direct measurement (7.37). Again, Arunlakshana & Schild (1959) have shown that for competitive antagonism the value of log K_2 should agree with the pA_2 value: in the present experiments the mean of three values of log K_2 was 7.82 and thus close to the pA_2 of 7.54.

The accepted criteria for simple competitive antagonism have therefore been fulfilled for thymoxamine antagonism of noradrenaline on human isolated arterial strips and the pA_2 values obtained are in reasonable agreement with those obtained on arterial strips from guinea-pigs (7.20), rabbits (6.90) and dogs (7.01) by Birmingham & Szolcsányi (1965), a finding which is pertinent in view of the theoretically required correspondence of these values between species. While it does not follow that *in vitro* proof of competition is proof of the same mode of action *in vivo*, the experiments previously reported on conscious cats did suggest that the action of thymoxamine in antagonizing the blood-pressure raising effect of noradrenaline has, at least in part, some of the characteristics of competition (Birmingham, Akubue & Szolcsányi, 1967).

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