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RECEPTORS FOR NORADRENALINE AND HISTAMINE IN THE RABBIT'S POSTERIOR VENA CAVA

BY

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Although the responses of isolated venous strips to several vasoactive substances and the modification of these responses by antagonists have been studied (Sutter, 1965), data on the quantitative aspects of such antagonism are not available. We have examined the effects of some vasoactive substances on the post caval venous strips of rabbits and made a quantitative study of the antagonistic effect of blocking drugs. Some experiments using the "receptor protection" technique of Furchgott (1954) were also performed. It was expected that the data on the quantitative aspects of antagonism and those obtained with the "receptor protection" experiments would yield information about the receptors in the veins.

METHODS

Strips were obtained from the posterior vena cava of young rabbits of either sex, killed by a blow on the head and prepared in the manner described by Sutter (1965). Spirally cut strips 3–3.5 cm long and 2–3 mm wide were suspended in a 40-ml. organ bath containing Krebs bicarbonate solution of the following composition (g/l. of distilled water): NaCl, 6.88; KCl, 0.42; anhydrous $CaCl_2$, 0.28; anhydrous $MgCl_2$, 0.112; NaH_2PO_4, 0.18; $NaHCO_3$, 2.1; and dextrose, 1.8. The solution was maintained at 37°–38° C and 5% carbon dioxide in oxygen was bubbled through it in both the bath and the reservoir. The pH of the solution was then 7.4. The strips were suspended in the bath for at least 30 min before study. Isotonic contractions at 0.5 g tension were recorded on a smoked drum at ten times amplification.

Dose-response curves for noradrenaline (6.5×10^{-8}, 1.3×10^{-7}, 2.6×10^{-7}, 5.2×10^{-7} and 1.04×10^{-6}) and for histamine (1.25×10^{-7}, 2.5×10^{-7}, 5×10^{-7}, 1×10^{-6} and 2×10^{-6}) were obtained by the cumulative administration of increasing concentrations of the amines, allowing the contraction to develop fully after each administration (2 min for noradrenaline and 2.5 min for histamine). In individual experiments the height of maximal contraction was first determined by the cumulative addition of the amines and the preparation was then repeatedly washed for 60 min. Cumulative dose/response curves for noradrenaline and histamine with responses between 20 and 80% of the maximal were then determined at intervals of 45 min on each strip before the administration of the antagonist (phentolamine or antazoline). In studies on antagonism, the antagonist was kept in the bath for 20 min before the addition of the agonist and remained in the bath thereafter.

An attempt was also made to obtain contractile responses of the venous strip to several other known *in vitro* vasoactive substances, such as 5-hydroxytryptamine, acetylcholine, angiotensin and bradykinin. If a given agent elicited a contraction, several known antagonistic drugs were used to obliterate the response.

The technique of experiments on "receptor protection" was essentially similar to that described by Furchgott (1954). After eliciting reproducible responses to the agonist (noradrenaline or histamine), the strip was exposed to a high concentration of the same agonist (5×10^{-6}). At the peak of contraction, the strip was exposed to phentolamine or antazoline for 10 min after which the strip was washed. Fifteen minutes later the strip was washed again and after a further 15 min the strip was tested for its sensitivity to standard doses of noradrenaline or histamine (protection experiments). The same strip was then repeatedly washed and when responses to standard doses of noradrenaline or histamine were fully restored, it was exposed to antagonist (phentolamine or antazoline) alone for 10 min, washed as before and again tested 30 min after removing the antagonist (control experiments).

Drugs

The drugs used were: noradrenaline bitartrate, histamine acid phosphate, acetylcholine, 5-hydroxytryptamine creatinine sulphate, angiotensin (hypertensin, Ciba), bradykinin (BRS 640, Sandoz), phentolamine methane sulphonate, antazoline methane sulphonate, and acetylsalicylic acid. Drug concentrations are expressed as g/ml.

RESULTS

Responses of the rabbit vena caval strip to noradrenaline, histamine, 5-hydroxytryptamine, acetylcholine, angiotensin and bradykinin

Noradrenaline and histamine. Cumulative administration of increasing concentrations of noradrenaline or histamine elicited increasing contractile responses from the vena caval strips and although the sensitivity of individual strips to noradrenaline or histamine varied somewhat, statistically linear dose/response relationships were obtained when the percentage of maximal contraction was plotted against the log dose of the agonist.

Angiotensin and bradykinin. Angiotensin (1×10^{-7} to 4×10^{-6}) given at 30 min intervals produced dose-related contractile responses. It was not possible to obtain cumulative dose/response curves because if angiotensin was given at shorter intervals tachyphylaxis occurred. Bradykinin (1.25×10^{-6} to 1×10^{-5}) given at 30 min intervals produced dose-related contractile responses. Administration of bradykinin at shorter intervals led to tachyphylaxis.

Acetylcholine and 5-hydroxytryptamine. Acetylcholine (1×10^{-7} to 1.6×10^{-6}) and 5-hydroxytryptamine (1×10^{-8} to 1×10^{-5}) did not elicit any contractile response.

Antagonism studies

In preliminary experiments it was found that a contact time of less than 20 min was insufficient for optimum block by phentolamine or antazoline.

Noradrenaline-phentolamine. When two successive dose/response curves for noradrenaline were alike, the preparation was rested for 25 min and then exposed to phentolamine for 20 min before the addition of noradrenaline, so that the interval between the two successive determinations was always kept constant. Phentolamine (7.5×10^{-9} to 2×10^{-7}) caused a parallel shift to the right of the dose/response lines for noradrenaline (Fig. 1a) suggesting a competitive type of antagonism. The degree of antagonism was expressed in terms of the "dose ratio". The logarithm of the dose ratio is given by the horizontal distance between the parallel lines. In fifteen experiments dose ratios for five different concentrations of phentolamine were determined in triplicate with noradrenaline (three sets of experiments).

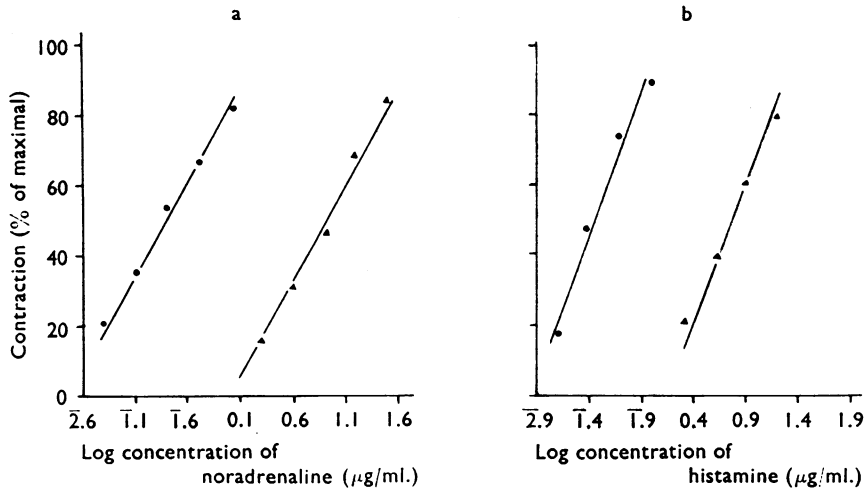

Fig. 1. Rabbit posterior vena caval strip. Cumulative log dose/response curves (a) for noradrenaline alone (●——●) and in the presence of 1×10^{-7} of phentolamine (▲——▲) and (b) for histamine alone (●——●) and in the presence of 1.6×10^{-5} of phentolamine (▲——▲). Phentolamine causes a parallel shift of the dose/response curve to the right. The horizontal distance between the two curves in (a) or (b) gives the logarithm of the ratio of the doses of noradrenaline or histamine respectively causing equal contractions in the presence and in the absence of the inhibitor (phentolamine).

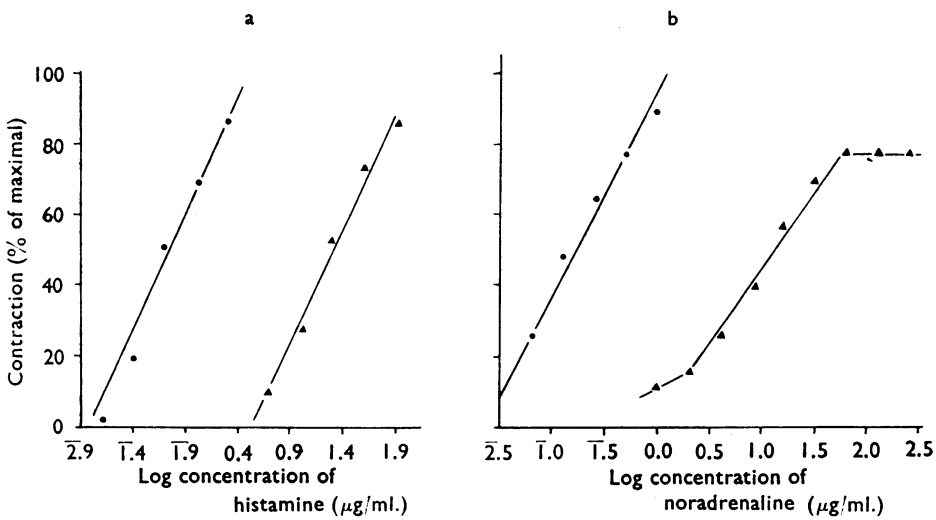

Fig. 2. Rabbit posterior vena caval strip. Cumulative log dose/response curves (a) for histamine alone (●——●) and in the presence of 4×10^{-7} antazoline (▲——▲) and (b) for noradrenaline alone (●——●) and in the presence of 3.2×10^{-5} antazoline (▲——▲). Antazoline causes a parallel shift of the dose/response curve for histamine to the right and the horizontal distance between the two curves gives the dose ratio (the logarithm of the ratio of doses of histamine causing equal contractions in the presence and in the absence of antazoline). In the case of noradrenaline the slope of the dose/response curve and the maxima are depressed by antazoline. The horizontal distance between the two curves at 50% of the maximal contraction gives the dose ratio.

Because a parallel shift to the right of the dose/response line for agonist by antagonist does not provide complete proof of competitive antagonism, the present results were subjected to further analysis to see whether they fitted the hypothesis of competitive antagonism. The logarithm of (x-1) was plotted against the negative logarithm of B, where x is the dose ratio and B is the corresponding molar concentration of phentolamine. Results of three sets of experiments were plotted separately. A representative plot for phentolamine is shown in Fig. 3a where the line is the calculated regression line. The line is significantly different from zero ($P<0.001$). The intercept of this line with the abscissa (at zero level) gave the pA_2 value for phentolamine. The pA_{10} value was determined from the regression line. The mean pA_2 value for phentolamine in antagonizing responses to noradrenaline was 8.0 ± 0.21. The mean value of pA_2–pA_{10} was 0.90. In order to test whether the observed value of pA_2–pA_{10} was significantly different from the theoretical value (0.95) of pA_2–pA_{10}, the method suggested by Marshall (1955) was employed. The theoretical value (0.95) of pA_2–pA_{10} was added to each pA_{10} value and then by applying the "t" test it was determined whether this sum differed from the pA_2 value. The difference between the pA_2 value for phentolamine and the sum was not statistically significant ($P>0.5$).

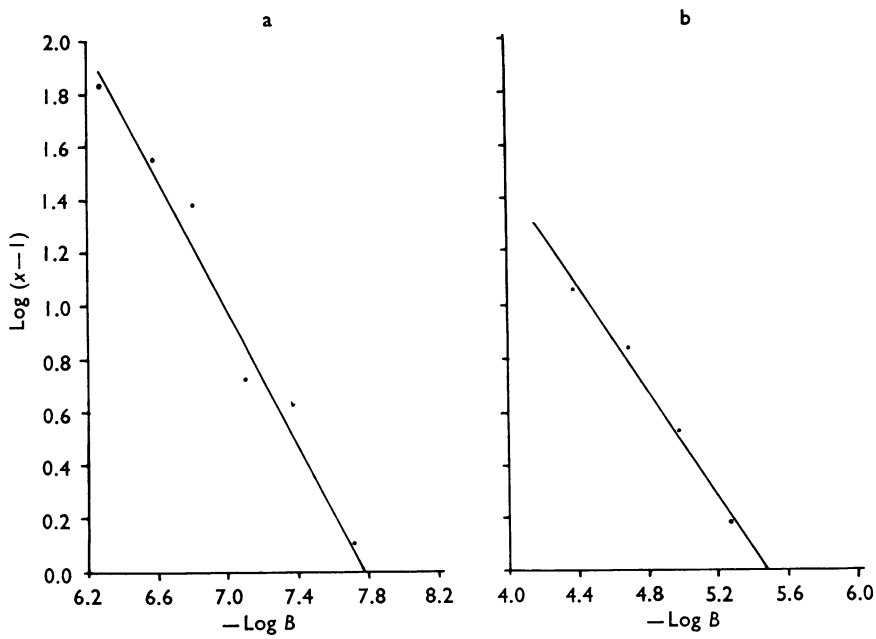

Fig. 3. Results of one set of experiments with the rabbit posterior vena caval strip preparations plotted by the method of Arunlakshana & Schild (1959) to determine the values of pA_2–pA_{10}. Ordinate: log (x-1) where x is the ratio of equiactive doses of noradrenaline (in a) or histamine (in b) in the presence and in the absence of phentolamine. Abscissa: negative log B where B is the molar concentration of phentolamine. The best fitting straight lines through the plotted points were determined by regression analysis and are very highly significant ($P<0.001$). The lines intersect the abscissa at 7.78 (in a) and at 5.48 (in b) which are the pA_2 values for antagonism of noradrenaline and histamine respectively. The individual values of pA_2–pA_{10} are 0.76 (in a) and 0.95 (in b). (Values given in the text are mean values.)

Histamine-phentolamine. Phentolamine was used in concentrations ranging from 2×10^{-6} to 1.6×10^{-5}. The analysis of data on histamine-phentolamine antagonism was similar to that detailed above for noradrenaline-phentolamine antagonism (Figs. 1b and 3b; twelve experiments). The mean pA_2 value for phentolamine in antagonizing responses to histamine was 5.44 ± 0.066. The value of pA_2–pA_{10} was 0.89. This value is not significantly ($P>0.1$) different from the theoretical value.

Histamine-antazoline. The design of experiments and the way the results were analysed were similar to those described for noradrenaline-phentolamine antagonism (Fig. 2a; twelve experiments). Dose ratios for four different concentrations of antazoline (5×10^{-8} to 4×10^{-7}) were determined in triplicate with histamine and the value of log (x-1) for each concentration of antazoline was used for further analysis (Fig. 4a). The mean pA_2 value for antazoline in antagonizing responses to histamine was 7.37 ± 0.015. The value of pA_2–pA_{10} for antagonism of histamine responses was 0.86. The value obtained in the present experiments is not significantly ($P<0.1$) different from the theoretical value of 0.95 for competitive antagonism.

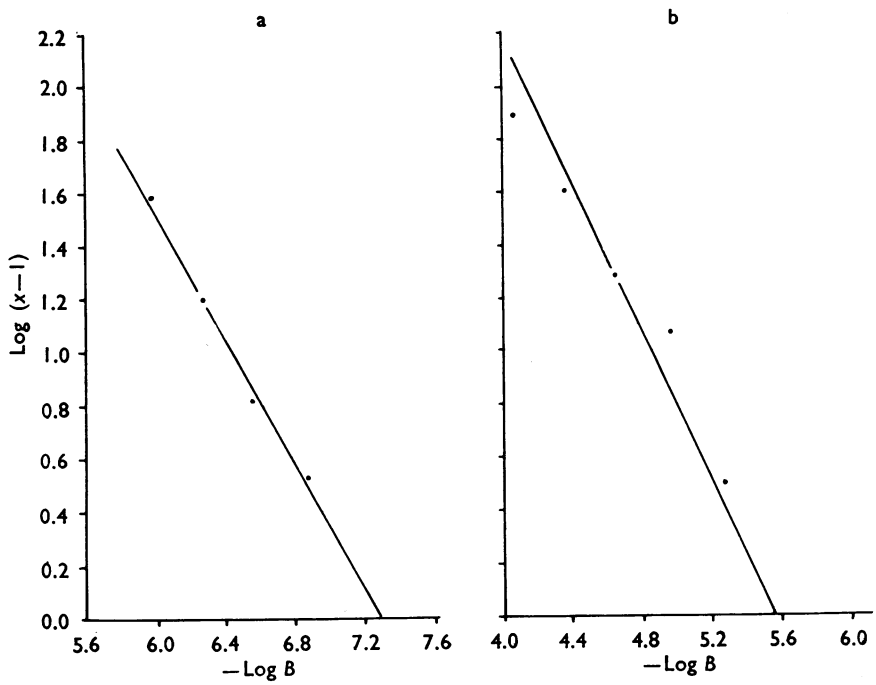

Fig. 4. Results of one set of experiments with the rabbit posterior vena caval strip preparations plotted by the method of Arunlakshana & Schild (1959) to determine the values of pA_2 and pA_2–pA_{10}. Ordinate: log (x-1) where x is the ratio of equiactive doses of histamine (in a) or noradrenaline (in b) in the presence and in the absence of antazoline. Abscissa: negative log B where B is the molar concentration of antazoline. The best fitting straight lines through the plotted points were determined by regression analysis and are very highly significant ($P<0.001$). The lines intersect the abscissa at 7.29 (in a) and 5.56 (in b) which are the pA_2 values respectively for antagonism of histamine and noradrenaline responses. The individual value of pA_2–pA_{10} in (a) is 0.81 and in (b) it is 0.62. (Values given in the text are mean values.)

Noradrenaline-antazoline. Antazoline in small doses (2×10^{-6} to 8×10^{-5}) caused a parallel shift of the dose/response curve for noradrenaline to the right, but in higher doses (1.6×10^{-5} and 3.2×10^{-5}) the maximum response was reduced (Fig. 2b). The data suggest that at lower doses the antagonism is competitive but at higher doses it is not competitive. In fifteen experiments dose ratios for five different concentrations of antazoline were determined in triplicate. For parallel lines, log of the dose ratio was

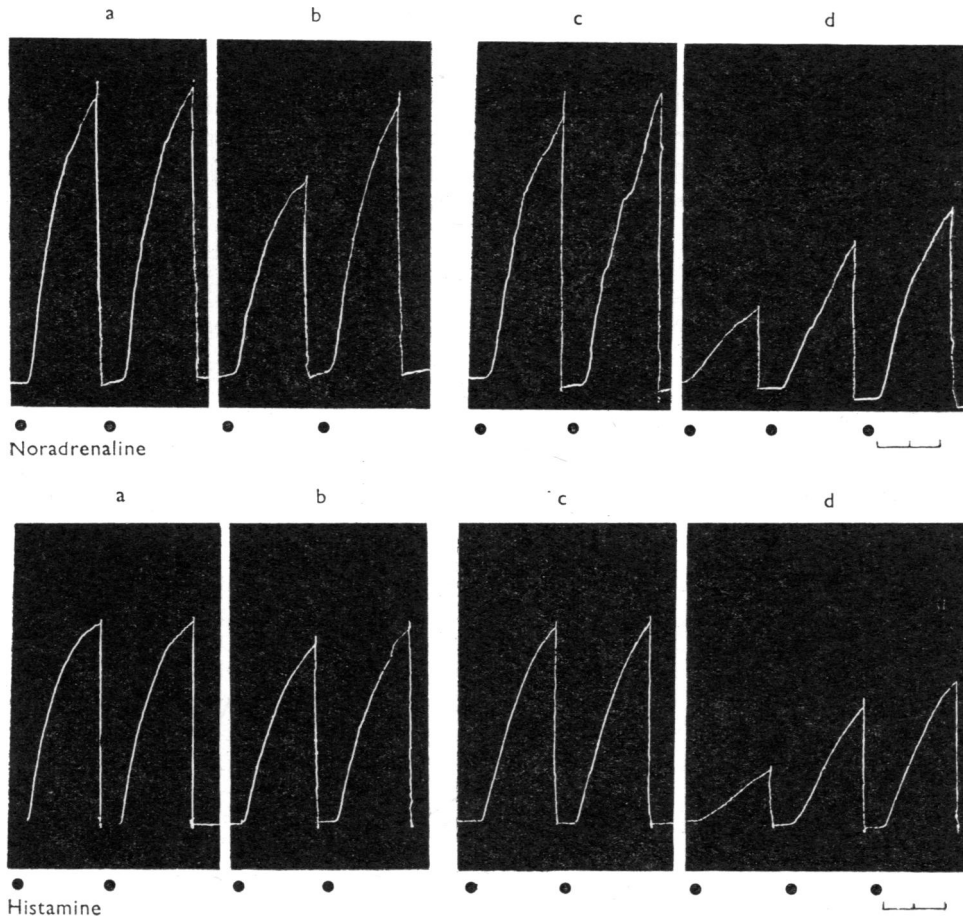

Fig. 5. Rabbit posterior vena caval strip preparations. The records show responses of the protected (a and b) and the unprotected (c and d) strips to noradrenaline (5×10^{-8}, upper record) and to histamine (5×10^{-7}, lower record) before (a and c) and 30 min after a 10 min exposure to antagonist (b and d). In the upper records the strip was exposed twice to the same concentration (1×10^{-7}) of phentolamine but on the first occasion it was protected (between a and b) by a high concentration (5×10^{-6}) of noradrenaline which was placed in the bath 6 min before the addition of phentolamine. In the lower records the strip was exposed twice to the same concentration (2×10^{-7}) of antazoline but on the first occasion it was protected (between a and b) by a high concentration of histamine (5×10^{-6}) which was placed in the bath 6 min before the addition of antazoline. Time mark, 1 min.

given by the horizontal distance between the parallel lines, and for non-parallel lines it was calculated from the horizontal distance between the dose/response curves at 50% of the maximal response. Further analysis of the results was similar to that detailed for noradrenaline-phentolamine antagonism (Fig. 4b). The mean pA_2 value for antazoline was 5.62 ± 0.068. The value of pA_2–pA_{10} was 0.70. This value is significantly ($P<0.05$) lower than the theoretical value of 0.95 suggested for competitive antagonism.

Miscellaneous. Phentolamine (2×10^{-7} and 2×10^{-5}) failed to affect the responses of the posterior caval strips to bradykinin or angiotensin. Acetylsalicylic acid (2×10^{-4}) did not block responses to bradykinin.

Receptor protection

In receptor protection experiments it was observed that when the strips were protected by a high concentration of agonist (noradrenaline or histamine) considerable to full responses to the agonist continued to be elicited despite the presence of the antagonist. On the other hand, when the preparation was tested after 1 hr of repeated washing in the presence of the same concentration of the antagonist, the responses were considerably blocked (Fig. 5). Moreover, recovery from block was slow when the preparation was not protected by a high concentration of agonist. This was rather unexpected since competitive blockers leave the receptors sufficiently rapidly to allow the agonist to act. The slow rate of recovery could be the result of slow rate of escape of phentolamine from the "biophase" as suggested by Furchgott (1955). That a contact time of less than 20 min was insufficient for optimum block by phentolamine or antazoline further supports this suggestion.

DISCUSSION

Our results show that antagonism by phentolamine of responses to noradrenaline and histamine, and by antazoline of responses to histamine, fulfil the criteria for competitive antagonism (Arunlakshana & Schild, 1959). Thus (1) the antagonists in a wide range of concentrations caused a parallel shift of the dose/response lines, and (2) the values of pA_2–pA_{10} for noradrenaline: phentolamine antagonism (0.90), for histamine: antazoline antagonism (0.86) and for histamine: phentolamine antagonism (0.89) are closely similar to the theoretical value of 0.95 for competitive antagonism.

Antagonism by antazoline of responses to noradrenaline does not fulfil the criteria for competitive antagonism because at higher concentrations of antazoline there was a definite flattening of the dose effect curves; the maximal response was reduced in all the preparations, and the pA_2, pA_{10} difference was significantly lower than 0.95. This is in agreement with the conclusion drawn earlier by Sethi, Gulati, Gokhale & Joseph (1967) regarding the nature of antagonism by antazoline against noradrenaline on the rabbit aortic strip and the rat seminal vesicle.

The pA_2 value described by Schild (1947) is a precise and generally accepted measure of the potency of an antagonist. Agonists which act at the same receptors can theoretically be expected to produce the same pA_x with a competitive antagonist. The pA_x value can also be used to identify the receptors in different tissues, since tissues with similar receptors would be expected to give the same pA_x for a given antagonist. Thus the mean pA_2 value for phentolamine against noradrenaline obtained in the present study

(8.00) is similar to that reported by Furchgott (1955) with rabbit aortic strip (7.52). The mean pA_2 values for antazoline and phentolamine using histamine as agonist were 7.37 and 5.44 respectively. The pA_2 values for antazoline (with histamine as agonist), with guinea-pig ileum, is 7.55 as reported by Reuse (1948) and 7.67 as reported by Marshall (1955). The pA_2 value reported for phentolamine against histamine on the rabbit aortic strip is 5.0 (Furchgott, 1955). Thus the pA_2 values obtained in our experiments are closely similar to those reported in other tissue preparations. These results would indicate that receptors for noradrenaline and for histamine in the rabbit posterior vena cava are fairly similar to those in the other tissue preparations.

The " receptor protection " experiments offer evidence that the antagonist (phentolamine or antazoline) inhibits the response to a given agonist (noradrenaline or histamine) by blocking the receptors with which the agonist itself combines.

Collier, Holgate, Schachter & Shorley (1959, 1960) found that bradykinin causes bronchoconstriction in the guinea-pig; that small doses of acetylsalicylic acid, phenylbutazone and amidopyrine suppress this response without affecting the responses to histamine, 5-hydroxytryptamine or acetylcholine, and that increasing doses of bradykinin overcome this suppression. Acetylsalicylic acid, phenylbutazone and amidopyrine did not, however, specifically antagonize the action of bradykinin on the capillaries of guinea-pig skin *in vivo*, on guinea-pig ileum *in vitro* or on rat duodenum *in vitro* (Collier & Shorley, 1960). It has been observed that hexamethonium or phentolamine (in low concentration) either of which blocks acetylcholine vasoconstriction in the rabbit ear, cannot block acetylcholine contraction in the rabbit aortic strip (Furchgott, 1955). In the present experiments acetylsalicylic acid failed to affect the responses to bradykinin. This suggests that acetylsalicylic acid specifically antagonizes bradykinin in its bronchoconstrictor action but not in its other actions. Such differences may be attributed to the existence of different types of receptors for bradykinin as is true for acetylcholine.

Nickerson & Sutter (1964) and Zimmerman, Abboud & Eckstein (1964) reported the venoconstrictor action of angiotensin *in vivo* whereas Folkow, Johansson & Mellander (1961) failed to detect a venoconstrictor effect *in vivo* in response to angiotensin. Sutter (1965) reported that angiotensin could contract both circular and longitudinal muscle of the rabbit external jugular, posterior caval and anterior mesenteric vein. The failure of previous workers to detect venoconstriction by angiotensin *in vivo* may be attributed to the rapidity with which the response to angiotensin fades despite the continued presence of the peptide. In the present experiments it was not possible to obtain a reproducible cumulative dose/response curve for angiotensin, but if angiotensin was given in single doses at 30 min intervals (1×10^{-7} to 4×10^{-6}) then dose-related contractile responses were elicited. This is probably the result of the development of tachyphylaxis or to a rapid fading away of the response.

SUMMARY

1. Cumulative administration of increasing concentrations of noradrenaline or histamine elicited increasing contractile responses from isolated posterior vena caval strips of rabbits.

2. Angiotensin and bradykinin given at 30 min intervals produced dose-related contractile responses. It was not possible to obtain cumulative dose/response curves with

angiotensin and bradykinin because if they were given at shorter intervals tachyphylaxis occurred.

3. Acetylcholine and 5-hydroxytryptamine did not elicit any contractile response.

4. Antagonism by phentolamine of the contractile responses to noradrenaline and histamine fulfilled the conditions of competitive antagonism.

5. Antagonism by antazoline of the contractile responses to histamine fulfilled the conditions of competitive antagonism. Antagonism by antazoline of the contractile responses to noradrenaline did not fulfil the conditions of competitive antagonism.

6. Phentolamine failed to affect responses of the posterior caval strips to bradykinin or angiotensin. Acetylsalicylic acid did not block responses to bradykinin.

7. A high concentration of noradrenaline or histamine protected the strips from block by phentolamine or antazoline respectively.

8. It is concluded that the receptors for noradrenaline and histamine in the rabbit posterior vena cava exhibit a fair degree of similarity to those in other tissues.

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