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RESPONSES OF RABBIT PORTAL VEIN TO HISTAMINE

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1 Histamine produced a dose-dependent contraction of the isolated portal vein of the rabbit. This contraction was not antagonized by atropine, methysergide, indomethacin, cocaine or 6-hydroxydopamine, nor by pretreatment of the rabbit with reserpine.

2 The response to histamine was blocked by H_1-receptor antagonists only when the blocking agent was used in very high concentrations, and was not antagonized by the H_2-receptor blocking agent, metiamide. H_1-receptor antagonists did not block the effects of 5-hydroxytryptamine.

3 The contractions elicited by histamine, 5-hydroxytryptamine and noradrenaline were blocked by phentolamine.

4 Desensitization to high doses of 5-hydroxytryptamine caused a concomitant depression in the response to histamine but not to noradrenaline or acetylcholine.

5 The results suggest that the contractions of rabbit portal vein elicited by histamine are not mediated by receptors of the H_1- or the H_2-type, but may involve an action of histamine at a receptor which is also involved in the action of 5-hydroxytryptamine.

Introduction

While many of the large arteries are known to produce a contraction to a variety of different agents (Somlyo & Somylo, 1970), veins are generally much less responsive. A significant exception to this general rule is the portal vein (Sutter, 1965). This vessel is known to contract *in vitro* to acetylcholine, histamine, noradrenaline, 5-hydroxytryptamine and electrical stimulation (Sutter, 1965; Hughes & Vane, 1967). Although the ultrastructure and electrophysiological properties of portal vein have been extensively studied (Hughes & Vane, 1967; Ljung, 1970), comparatively few pharmacological studies have been carried out. The isolated portal veins of rabbit and guinea-pig are reported to respond to histamine over a dose range of 10^{-6} M to 5×10^{-5} M (Hughes & Vane, 1967), but it is not clear whether this response is caused by a direct action on the muscle or by an indirect action arising from histamine-induced release of an endogenous spasmogen. It is also uncertain whether the response is mediated by H_1- or H_2-receptors, both of which are involved in the cardiovascular responses to histamine of the intact animal (Black, Owen & Parsons, 1975).

Because of the importance of the portal system to the circulatory system as a whole, and the increasing interest in the cardiovascular effects of histamine, attempts have been made to characterize the responses to histamine of isolated strips of rabbit portal vein.

Methods

New Zealand white rabbits were killed by cervical dislocation. The hepatic portal vein was immediately dissected free from the surrounding mesentery and placed in warm oxygenated Krebs solution. This vein was then cleaned and cut into a spiral strip. The spiral strip was divided into 4 parallel pieces along the longitudinal axis. These were suspended under 5 g tension in isolated tissue baths, with a working volume of 28 ml, containing Krebs solution, maintained at 37°C and bubbled with 95% oxygen and 5% CO_2. Contractions were recorded isotonically with Hewlett Packard 7DCDT transducers connected to a Grass Polygraph (Model 5PI).

The tissues were allowed to equilibrate for 1 h before their responses to a maximum dose of potassium chloride (10^{-1} M) were obtained. Thereafter, cumulative dose-response curves to histamine were obtained at 60 min intervals over 5 h, except when antagonists were added, in which case the time was suitably adjusted for the onset and offset of antagonism. Where more than one concentration of an-

tagonist was added to a tissue, a control response to histamine alone was taken before the second dose of antagonist was added, in order to ensure that complete recovery from blockade had taken place.

Reserpine-treated tissues were obtained from animals which had received reserpine 1.0 mg/kg subcutaneously 16 h and reserpine 0.5 mg/kg intravenously 1 h before they were killed.

In the experiments conducted with 6-hydroxydopamine (6-OHDA), this agent was added to the bath at a concentration of 250 μg/ml, together with ascorbic acid 1.1 mg/ml (Wadsworth, 1973). After 15 min the tissues were washed with normal Krebs solution until constant responses to histamine and acetylcholine were obtained.

In order to induce tachyphylaxis to tyramine, a control response to histamine and tyramine was first obtained, and the tissues were then treated with repeated doses of tyramine at 3 min intervals, washing 4 times between each dose. When the response to tyramine was reduced to less than 35% of the initial value, a second response to histamine was obtained. A similar approach was used to induce desensitization to 5-hydroxytryptamine.

The following drugs were used: histamine dihydrochloride (Sigma), (−)-noradrenaline (norepinephrine) bitartrate (Sigma), acetylcholine bromide (BDH Ltd.), tyramine hydrochloride (Mann Research), 5-hydroxytryptamine (Sigma), chlorpheniramine maleate (Schwarz/Mann Co.), diphenhydramine hydrochloride (Sigma), phentolamine methansulphonate (Ciba), metiamide (SKF), methysergide (SKF), 6-hydroxydopamine hydrobromide (Regis), cocaine hydrochloride (BDH Ltd.), L-ascorbic acid (MCB) and reserpine (Sigma).

Results were analysed for significance by Student's paired or unpaired t tests.

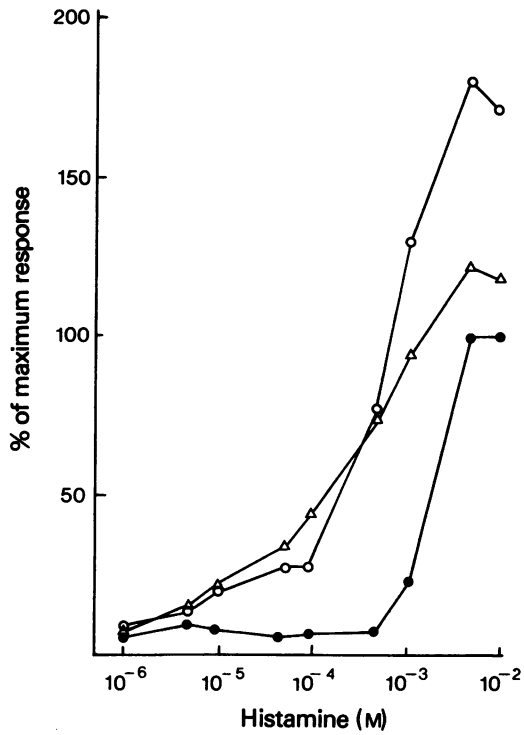

Figure 1 Responses of rabbit portal vein to histamine. Molar concentrations are shown on abcissa scale, percentage of maximum response on ordinate scale. $n = 14$. (●) Dose-response curve after 1 h equilibration; (○) dose-response curve in normal tissues after 5 h; (△) dose-response curve in reserpine-treated tissues after 5 hours.

Results

Responses of portal vein to histamine in normal and reserpine-treated tissues

Histamine produced dose-dependent contractions of the isolated spiral strips, with a threshold at 10^{-6} M and a maximum at 5×10^{-3} M (Figure 1). Concentrations greater than 5×10^{-3} M resulted either in no increase or a slight decrease in the magnitude of the maximum contraction. When dose-response curves were obtained at 60 min intervals over a period of 5 h, it was found that the response of the tissue to histamine was increased by up to 75% of the initial maximum response (Figure 1). Control dose-response curves obtained with acetylcholine and noradrenaline over the same period indicated that only a small amount of sensitization occurred with acetylcholine and none with noradrenaline. However, the response to 5-hydroxytryptamine was also found to sensitize in a similar manner to that of histamine. Histamine also elicited contractions of the reserpine-treated portal vein (Figure 1). However, the amount of sensitization produced to histamine in the reserpine-treated portal vein over 5 h was considerably less than that produced in untreated tissue (Student's unpaired t test, $P < 0.05$), reaching an increase of only 25% of the initial maximum response.

Low doses of tyramine, a sympathomimetic amine which acts by causing noradrenaline release, also produced a response in reserpine-treated portal vein, suggesting that both histamine and tyramine could be acting by similar mechanisms. However, further investigation showed that no cross tachyphylaxis could be established between histamine and tyramine, the tyramine response being reduced to as little as 21% of control following repeated administration without

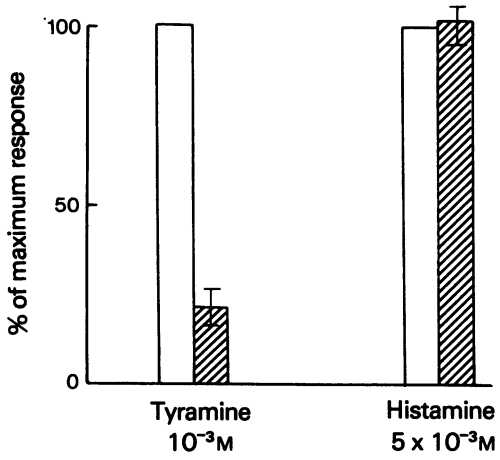

Figure 2 Histogram showing the effect of tyramine tachphylaxis on the responses to tyramine and histamine of rabbit portal vein. Percentage of maximum response shown on ordinate scale $n = 4$. Bars represent s.e. mean. Open columns: control responses; hatched columns: responses after tachyphylaxis to tyramine had been induced.

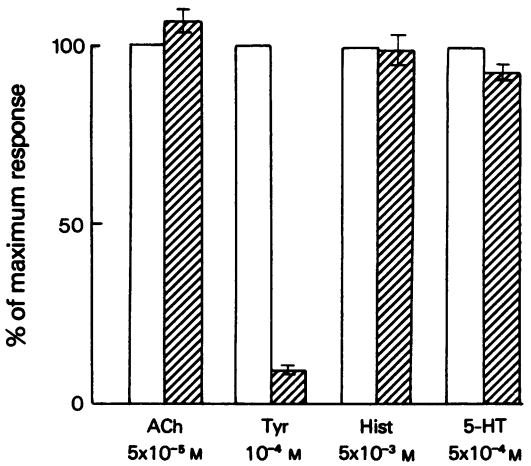

Figure 3 Histogram showing the effect of 6-hydroxydopamine on the responses to acetylcholine (ACh), tyramine (Tyr), histamine (Hist) and 5-hydroxytryptamine (5-HT) of rabbit portal vein. Percentage of maximum response on ordinate scale. $n = 6$. Bars represent s.e. mean. Open columns: control responses; hatched columns: responses after 6-hydroxydopamine (250 μg/ml, for 15 minutes).

affecting the response to histamine (Figure 2). The addition of 6-hydroxydopamine, an agent known to destroy adrenergic nerve terminals (Malmfors & Sachs, 1968), completely eliminated the response to tyramine 10^{-4} M without specifically affecting the response to histamine or to 5-hydroxytryptamine (Figure 3). Similarly, cocaine 1.7×10^{-5} M reduced the response to tyramine 10^{-5} M to 7% of control, and to tyramine 10^{-4} M to 43% of the control value, without affecting the histamine response.

Blockade with atropine, indomethacin and methysergide

To establish further the nature of the response to histamine, indomethacin, a prostaglandin synthetase inhibitor, was added to the bath at a concentration of 3×10^{-6} M for up to 3 hours. This dose of indomethacin had no effect on either the magnitude of the contractions to histamine, nor on the increase in sensitivity to histamine over the 5 h time period. Increases in the concentration of indomethacin result in non-specific blockade of the tissue, possibly by interference with Ca^{2+} fluxes (Northover, 1972). Similarly, atropine, a muscarinic antagonist, given at a dose sufficient to shift the dose-response curve to acetylcholine two to three log units to the right (10^{-7} M), had no effect on the dose-response curve *per se* nor the sensitization to histamine.

The presence of methysergide (10^{-6} M) did not successfully antagonize the maximum response to either 5-hydroxytryptamine or histamine even after prolonged incubation with the antagonist. However, a significant ($P < 0.05$) reduction in the sensitization to both agonists was observed following methysergide treatment.

Blockade with H_1- and H_2-antagonists

Two H_1-antagonists, diphenhydramine and chlorpheniramine maleate, were used. Both agents blocked the response to histamine in this tissue (Figure 4), but were only effective at doses of 10^{-6} M or greater. The antagonism produced was specific for histamine, since the response to noradrenaline in the presence of these agents did not differ significantly from the control. The H_1-receptor blocking agents did not antagonize the responses to 5-hydroxytryptamine.

The H_2-antagonist, metiamide, was ineffective in antagonizing the response to histamine, except at concentrations greater than 10^{-4} M. The blockade produced did not appear to be histamine-specific, since these concentrations of metiamide also resulted in antagonism of the responses to both acetylcholine and noradrenaline.

Blockade with phentolamine

The α-adrenoceptor antagonist, phentolamine, was more effective in antagonizing the response to histamine than any of the anti-histamines employed. At

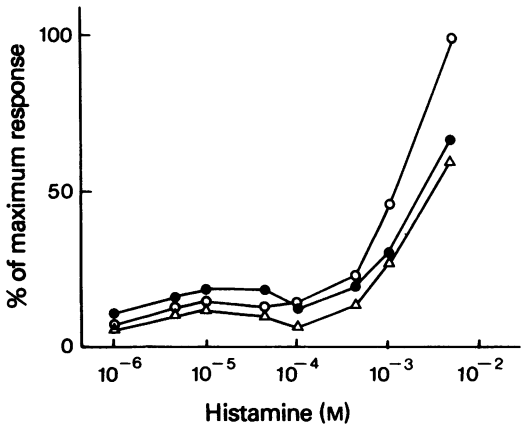

Figure 4 The effect of diphenhydramine and chlorpheniramine on the response to histamine of rabbit portal vein. Molar concentrations on abcissa scale, percentage of maximum response on ordinate scale. $n = 8$. (○) Control dose-response curve, corrected for sensitization; (△) dose-response curve after diphenhydramine 10^{-6}M; (●) dose-response curve after chlorpheniramine 10^{-6}M.

a concentration of 10^{-7} M, phentolamine caused a one log unit shift in the response to noradrenaline and reduced the maximum response to histamine to 40% of the control value (Figure 5). Phentolamine was also able to block the response to 5-hydroxytryptamine in this preparation, causing a one log unit shift in the response at a concentration of 10^{-7} M

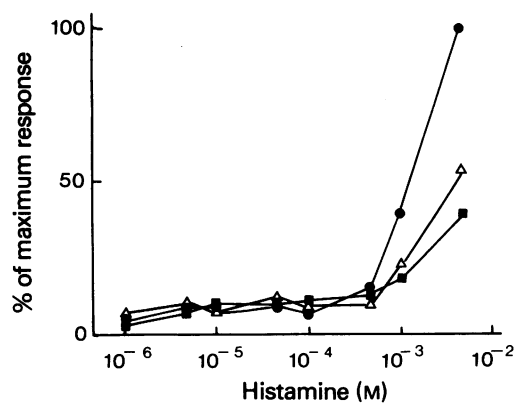

Figure 5 The effect of phentolamine on the response to histamine of rabbit portal vein. Molar concentrations on abcissa scale, percentage of maximum response on ordinate scale. $n = 8$. (●) Control dose-response curve, corrected for sensitisation; (△) dose-response curve after phentolamine 10^{-8}M; (■) dose-response curve after phentolamine 10^{-7}M.

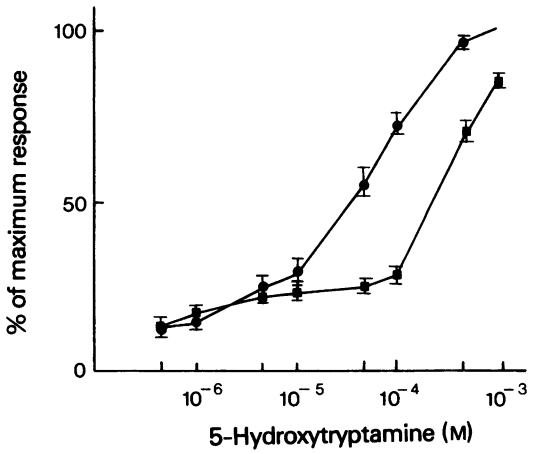

Figure 6 The effect of phentolamine on the response to 5-hydroxytryptamine of rabbit portal vein. Molar concentrations on abcissa scale, percentage of maximum response on ordinate scale. $n = 8$. Bars show s.e. mean (●) Control dose-response curve; (■) dose-response curve after phentolamine 10^{-7}M.

phentolamine. (Figure 6). Attempts to produce blockade with a second α-adrenoceptor antagonist, tolazoline, were unsuccessful, because tolazoline acted as an agonist in this preparation.

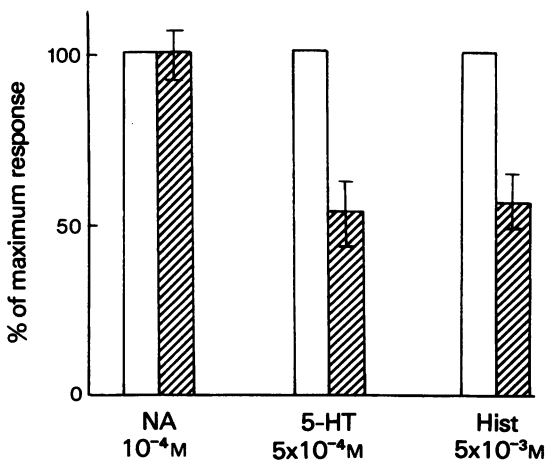

Figure 7 Histogram showing the effect of desensitization to 5-hydroxytryptamine on responses to noradrenaline (NA), 5-hydroxytryptamine (5-HT) and histamine (Hist) of rabbit portal vein. Percentage of maximum response on ordinate scale. $n = 8$. Bars represent s.e. mean. Open columns: control responses; hatched columns: responses after desensitization to 5-hydroxytryptamine had been induced.

Cross-desensitization

Exposure of the tissue to high doses of 5-hydroxytryptamine produced desensitization to this agonist. The responses to histamine but not to noradrenaline were diminished in tissues that had been desensitized with 5-hydroxytryptamine (Figure 7).

Discussion

Histamine produced a dose-dependent contraction of the isolated portal vein of the rabbit. Since this response was not affected by pretreatment of the tissue with reserpine, cocaine or 6-hydroxydopamine, the response is unlikely to result from an indirect action involving release of noradrenaline by histamine and is probably due to a direct action on smooth muscle.

Atropine did not produce significant antagonism of the histamine response, and thus it does not appear that there was a significant cholinergic component in the response. Indomethacin was ineffective at antagonizing the response to histamine suggesting that there was no prostaglandin involvement in either the magnitude of the contraction or the progressive sensitization of the tissue to histamine.

The dose-response curve produced by histamine in isolated portal vein differed from that seen in most other systems. Although the tissue responded to a threshold dose of 10^{-6} M histamine, it did not start to contract in a dose-dependent manner until a dose of 10^{-4} M histamine was reached. A maximum response occurred at 5×10^{-3} M. Since further increases in dose resulted in a slight decrease in the magnitude of the maximum contraction, it is possible that the tissue was becoming desensitized.

There was a significant increase in the sensitivity of portal vein to both histamine and 5-hydroxytryptamine over a period of up to 5 h, the maximum time over which responses were measured. This increase in sensitivity occurred only to a small extent with the response to acetylcholine and did not occur at all in the case of the noradrenaline-induced contractions. The mechanism responsible for the increase in sensitivity remains to be elucidated, although it may involve biogenic amines since either pretreatment with reserpine or the presence of methysergide significantly diminished this sensitization.

It has been reported previously that the histamine response in rabbit portal vein could be antagonized by mepyramine and antazoline, two classical H_1-antagonists (Sutter, 1965; Hughes & Vane, 1967). The present study has confirmed the observation that antihistamines can antagonize this response, but indicates that this antagonism takes place only at high doses of the blocking agents. Whether or not the antagonism is competitive or non-competitive is not clear, since the tissue did not respond to concentrations of histamine above 5×10^{-3} M in a reproducible manner, when the H_1-antagonists were present. Metiamide, an H_2-antagonist, was only effective at doses above 10^{-4} M. These caused depression of the response to acetylcholine and noradrenaline showing that, at these doses, metiamide is having a non-specific effect on the tissue, rather than acting as a true H_2-antagonist.

The responses to both 5-hydroxytryptamine and histamine were antagonized by the α-adrenoceptor blocking agent, phentolamine but not by either atropine or, surprisingly, methysergide. Very recently it has been reported that 5-hydroxytryptamine can interact with the α-adrenoceptor in rabbit ear artery and that this response is sensitive to phentolamine but not methysergide (Apperley, Humphrey & Levy, 1976). Further, it has been reported that phentolamine can antagonize the action of intraventricular histamine in rats (Finch & Hicks, 1975).

It is clear that the contractions elicited by histamine in rabbit portal vein were not mediated by typical H_1-(Ash & Schild, 1966) or H_2-receptors (Black, Duncan, Durant, Ganellin & Parsons, 1972; Black, Duncan, Conmett, Ganellin, Hesselbo, Parsons & Wylie, 1973). First, unusually high concentrations of the agonist are required, the H_1-receptor blocking drugs are weak antagonists of the response, and H_2-antagonists appear to be devoid of activity. Further, the sensitivity of the system to phentolamine is unusual for either receptor type, although the related compound tolazoline, which is an agonist in our system, is now known to have marked agonist effects at H_2-receptors (Saunders, Miller & Patil, 1975; Yellin, Sperow & Buck, 1975). The ability of phentolamine to antagonize the α-adrenoceptor and the responses of histamine and 5-hydrotryptamine in this system suggests the possibility that the effects of all these agonists are mediated by closely interrelated molecular events. The evidence is particularly strong for some correspondence between 5-hydroxytryptamine and histamine. Nevertheless, despite the cross-desensitization observed with these two agonists, high concentrations of the histamine H_1-antagonists block the histamine-induced contractions, but do not, at equivalent concentrations, alter the response to 5-hydroxytryptamine.

There are thus both properties in common and clear points of difference between the contractions elicited by noradrenaline, histamine and 5-hydroxytryptamine in rabbit portal vein. Despite the observation that the effects of histamine do not appear to be mediated by either receptor sub-type, it does not seem profitable to speculate that this tissue contains a third type of histamine receptor. Rather, it would appear that the responses are mediated by a receptor of rather low selectivity which can respond to a variety

of biologically active amines, although since some antagonists can select between the different agonists tested, presumably the nature of the binding of the agonists is not identical. It is also possible that separate receptors of each amine exist, but that they share a common mechanism for initiating the response following the drug-receptor interaction, and that desensitization and the action of phentolamine occur at this stage.

Finally, it should be pointed out that preliminary studies of the portal vein of the monkey suggest that the contractions elicited by histamine are mediated by a typical H_1-receptor. There is thus a species difference in the nature of the histamine receptors in this tissue, as has been found in other histamine-responsive tissues such as heart.

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