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Histamine H_1- and H_2-Receptors in Canine Renal Artery *in vivo* and *in vitro*

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The present work investigates (*a*) the modification by pretreatment with selective H_1- and H_2-receptor antagonists on the dose-response curves (DRC) to histamine for heart rate, blood pressure, renal arterial blood flow and renal vascular resistance in anesthetized dogs, and (*b*) the characteristics of the DRC to histamine in canine isolated renal artery. *In vivo*, pretreatment with metiamide (10 mg/kg i.v.) did not modify the DRC to histamine. In contrast, significant rightward shift of the DRC to histamine for all the hemodynamic parameters was observed after diphenhydramine (5 mg/kg i.v.). Combined pretreatment with metiamide and diphenhydramine resulted in further rightward displacement of the DRC to histamine. Analysis of the DRC to the relaxant effect of histamine in depolarized (K^+ 67 mM) isolated canine renal artery yielded an ED50 of 3.3×10^{-4} M and a Hill coefficient of 1.74. The results demonstrate the existence of the two types of histamine receptors, H_1 and H_2, in the renal artery of the dog, both mediating dilator responses, although the H_1-receptor appears to predominate.

The discovery by ASH and SCHILD (4) of two different populations of histamine receptors, named H_1 and H_2, prompted investigation on their involvement in the pharmacological and physiological actions of histamine.

The presence of histamine H_1- and H_2-receptors in the cardiovascular system and their relative contribution to the depressor response to this substance is well documented since the initial studies by BLACK *et al.* (8, 9), BRIMBLECOMBE (11), OWEN (28, 29) and OWEN and PARSONS (30). However, the systemic and local hemodynamic responses to histamine are very complex in nature and not yet fully understood as they reflect a complex balance between direct effects — depending on the distribution and activity of two different histamine receptors — and reflex changes in autonomic and renin-angiotensin systems triggered by variation in blood pressure. In addition, important species-dependent differences have been reported (2, 14, 20, 21, 27).

Recent experiments by JOHNSTON and

OWEN (21, 23) using radioactive microspheres to study the systemic hemodynamic and regional blood flow responses to i.v. administration of histamine in anesthetized cats indicated that (*a*) the dose-related fall in blood pressure caused by histamine was entirely due to a decrease in total peripheral resistance, and (*b*) the decrease in vascular resistance occurred predominantly in the heart, stomach and intestine with little vasodilatation elsewhere or even vasoconstriction in the spleen and skin.

Studies with selective agonists and antagonists of histamine receptors (18, 21-23) have demonstrated that histamine-induced coronary and gastrointestinal vasodilatation is mediated by both H_1- and H_2-receptors. A predominant role for the H_2-subtype in both territories has been reported by some authors (22, 23, 31) and for the H_1-subtype by others (18, 19, 25, 34). Less information is available about histamine receptor distribution in other regional circulations, probably because of the smaller magnitude of the histamine response.

Focussing on renal hemodynamics, the pharmacological effects of histamine still remain under investigation. In 1910, DALE and LAIDLAW (15), concluded that, conversely to the general vasodilatation caused by histamine, the kidney vasculature reacted with vasoconstriction. In 1928, MORIMOTO (26) also found renal vasoconstriction regardless of the dose of histamine administered to anesthetized cats and dogs. REUBI and FEUTCHER (33) observed decrease in renal plasma flow following subcutaneous administration of histamine to human. In contrast, BELL *et al.* (7), BLACKMORE *et al.* (10) and SINCLAIR *et al.* (39), reported increase in renal blood flow and decrease in renal vascular resistance after i.v. or i.a. administration of histamine in conscious and anesthetized dogs.

More recent experiments using radioactive microspheres demonstrated both increase (low doses) and decrease (high doses) of renal blood flow after i.v. infusion of histamine in anesthetized cats (18, 21). Constriction in kidney vasculature is probably due to the increased sympathetic tone and/or renin secretion (23) associated with the hypotension, whereas vasodilatation is a direct effect of histamine. Studies by the same authors (22, 23) with selective agonists and antagonists suggest that stimulation of both H_1- and H_2-histamine receptors produces renal vasodilatation but their relative contribution needs further clarification.

The present work was undertaken to investigate: the modification introduced by pretreatment with selective H_1- and H_2-antagonists on the dose-response curves to histamine for heart rate, blood pressure, renal blood flow and renal vascular resistance in anesthetized dogs, and the characteristics of the dose-response curve to histamine in canine isolated renal artery.

Materials and Methods

In vivo *experiments.* Mongrel dogs of either sex weighing 12 to 15 kg were anesthetized with sodium pentobarbital (30 mg/kg i.v.), intubated and placed on a positive pressure respirator pump. A femoral artery was cannulated and a catheter advanced into the abdominal aorta to measure arterial blood pressure by means of a HP 1280C pressure transducer connected to a HP 8805B carrier amplifier. The right external jugular vein was cannulated for drug administration. Heart rate was obtained from the pressure pulse using a rate computer HP 8812A.

A lateral abdominal incision gave access to the retroperitoneal space. The left main renal artery was identified and carefully dissected free for approximately 2 cm. Then, an appropriately sized (inside diameter 3 mm) electromagnetic flow transducer was placed around the exposed artery. Zero blood flow obtained period-

ically during the experiment by occlusion of the artery distal to the probe. Renal blood flow was measured with a Nycotron A/S376 electromagnetic flowmeter.

Renal flow (mean and phasic), arterial pressure (mean and phasic) and heart rate were recorded in a HP 7788A multichannel polygraph. Mean renal vascular resistance was calculated according to the formula: resistance (units) = mean aortic pressure (mmHg) ÷ mean renal arterial blood flow (ml/min).

Experimental design and data analysis. Three separate series of experiments were carried out. In the group A (6 dogs) control dose-response curves to histamine for heart rate, mean arterial blood pressure, mean arterial renal blood flow and renal vascular resistance were obtained by successive i.v. bolus injections of this drug from 0.125 to 4 μg/kg. A 10 min recovery period was allowed after each dose before the next was given. Thirty min after completion of the control dose-response curve to histamine, diphenhydramine (5 mg/kg) was administered i.v. and 15 min later a complete dose-response curve to histamine repeated. In group B (4 dogs) a similar protocol was followed, administering metiamide (10 mg/kg i.v.) instead of diphenhydramine. In group C (6 dogs) histamine dose-response curves were repeated after combined pretreatment with diphenhydramine (5 mg/kg) plus metiamide (10 mg/kg).

Peak hemodynamic responses were measured at each dose level and expressed as the mean ± S.E.M. of the absolute differences with respect to the resting value. Student's *t*-test was used to determine significance of differences between paired observations.

In vitro *experiments.* A segment of about 2 cm was removed from the main renal artery of anesthetized dogs (pentobarbital 30 mg/kg i.v.) and immediately immersed in oxygenated Krebs-bicarbonate solution at room temperature. The segment was cleaned of visible non-vascular tissue, cut into small intact cylindrical segments (around 7 mm of length) one of which was mounted between two metal holders in a 20 ml organ bath containing modified Krebs-bicarbonate solution (composition in g/l: NaCl 6.6, KCl 0.354, $CaCl_2$ 0.280, KH_2PO_4 0.161, $MgSO_4.7H_2O$ 0.145, $NaHCO_3$ 2.1 and dextrose 1) at 30° C and bubbled with 5 % CO_2 in oxygen.

Changes in tone of circular smooth muscle layers were recorded by an isometric transducer HP FTA 1001 connected to a carrier amplifier HP 8805B. A 60 min equilibration period under a resting tension of 1.5 g was permitted. Then, the cylinders were exposed to submaximal concentrations of potassium (67 mM) and after achieving a plateau level of muscle tone, cumulative dose-response curves to histamine were obtained. Doses of histamine ranged from 6.8×10^{-5} to 4×10^{-3} M. Histamine was freshly dissolved in Krebs-bicarbonate-potassium solution and added to the bath in volumes less than 10 % of the total volume of the bath. Only one dose-response curve was obtained from a single preparation.

The inhibition of the potassium-induced tone produced by each concentration was measured and expressed as the percentage (mean ± S.E.M.) of maximum relaxation elicited by histamine. An iterative procedure based on the modified Gauss-Newton method and the elimination of linear parameters (5) was applied to calculate the theoretical maximum effect and the 50 % effective dose (ED50).

Results

In vivo *experiments.* Histamine 0.125 to 4 μg/kg i.v. produced (fig. 1) dose-dependent increases in heart rate and decreases in blood pressure. Histamine responses in renal arterial blood flow were

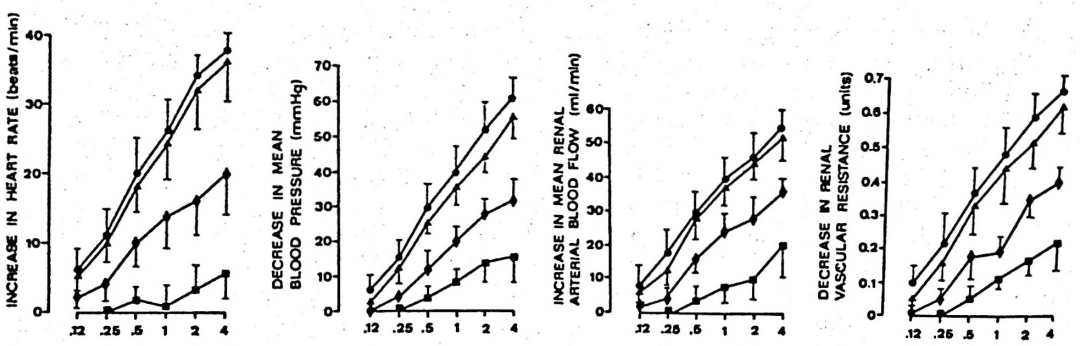

Fig. 1. *Influence of H_1- and H_2-receptor blockade on the responses to histamine in anesthetized dogs.*

Responses were obtained in a control situation (●), after metiamide 10 mg/kg i.v. (▲), after diphenhydramine 5 mg/kg i.v. (◆), and after combined pretreatment with metiamide 10 mg/kg i.v. and diphenhydramine 5 mg/kg i.v. (■). Abscissa is histamine dose in μg/kg.

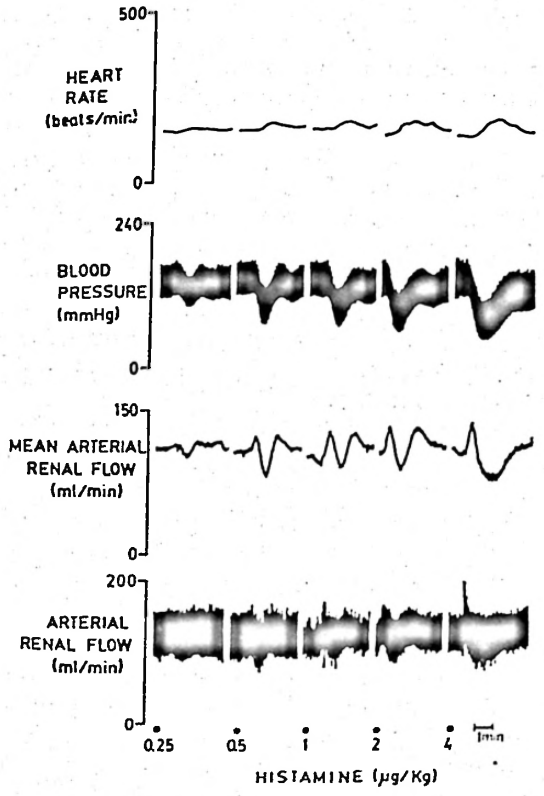

Fig. 2. *A typical recording showing heart rate, blood pressure and renal arterial blood flow changes after histamine.*

usually biphasic having an initial increase followed by a depression. A second peak in flow could be observed in some experiments (fig. 2). A similar pattern of changes in renal flow has been previously described (7).

The time-course of the events in renal blood flow after histamine may help to separate direct from reflex effects of this drug. The initial increase in flow (first peak in fig. 2) occurred before any change in heart rate or blood pressure and it can be considered as a direct effect of histamine. Conversely, only the initial direct increases in renal flow will be considered for the purpose of performing dose-response curves to histamine.

Pretreatment with metiamide (10 mg/kg i.v.) introduced no statistically significant modification of the dose-response curves to histamine (fig. 1). In contrast, a significant ($p < 0.01$) rightward shift of the dose-response curve to histamine for all the parameters considered was observed after diphenhydramine (5 mg/kg i.v.). Combined pretreatment with metiamide (10 mg/kg i.v.) plus diphenhydramine (5 mg/kg i.v.) resulted in further rightward displacement ($p < 0.01$) of the dose-response curve to histamine.

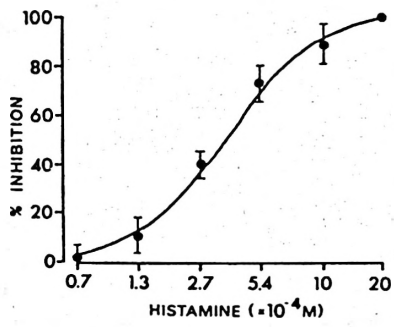

Fig. 3. *Theoretical log dose-response curve for relaxation by histamine in depolarized (K^+ 67 mM) isolated canine renal artery.* Experimental points (mean ± S.E.M.) are also plotted.

Separate or combined i.v. administration of the selective H_1- or H_2-receptor antagonists produced slight non-sustained decreases in arterial pressure and renal flow (probably secondary to the fall in perfusion pressure) and increases in heart rate (probably of reflex origin).

In vitro *experiments.* Analyses of the dose-response curve to the relaxant effect of histamine in isolated canine renal artery previously depolarized to a plateau by K^+ 67 mM yielded the following results (mean ± S.E.M. of 8 experiments) (fig. 3): $ED50 = 3.3 \times 10^{-4} \pm 0.5 \times 10^{-4}$ M, n (slope of the Hill plot) = 1.74 ± 0.22.

Discussion

The present results support the existence of two separate types of histamine receptors in the canine renal circulation and systemic vasculature.

The depressor response to histamine has been extensively studied. STAUB (40) and FOLKOW *et al.* (17) observed that the hypotensive response to large doses of histamine was refractory to H_1-antagonists. BLACK *et al.* (8) showed that refractory responses could be blocked by H_2-receptor blockers indicating involvement of both H_1- and H_2-receptors in these depressor responses.

In the present study, diphenhydramine caused a rightward shift of the dose-response curve to histamine for blood pressure, whereas metiamide alone failed to introduce any modification. However, combined pretreatment with diphenhydramine and metiamide further displaced to the right the dose-response curve to histamine. The failure of metiamide alone to displace the dose-response curve to histamine may be explained by applying (9) the theoretical model developed for an agonist (histamine) that interacts with two independent receptor systems (H_1- and H_2-histamine receptor populations) with a common effector system (vascular system) (3). Under these circumstances, ARIENS *et al.* (3) demonstrated that if the dissociation constant for the H_2-receptor is greater than the value for the H_1-receptor, the response to histamine is mainly determined by the interaction with H_1-receptors. The influence of H_2-receptor stimulation is unmasked after H_1-receptor blockade. This would explain both the failure of metiamide alone to modify histamine dose-response curves and the further displacement by metiamide of the blockade by diphenhydramine.

In consequence, the experimental evidence presented, along with other studies (8, 9, 11, 28-30), confirm the involvement of both H_1- and H_2-receptors in the fall of total peripheral resistance during intravascular administration of histamine. A predominant role in this response may be assigned to the H_1-receptors. Only ALBINUS and SEWING (1) and TUCKER *et al.* (41) reached the opposite conclusion although the interpretation has been criticized by JOHNSTON and OWEN (22).

Modifications in the heart rate dose-response curve to histamine in the presence of H_1-and/or H_2-antagonists closely resemble those observed for blood pressure. Similar results have been previously

reported (24, 32, 41). Caution is needed before interpreting these results as simply as activation of H_1- and H_2-receptors in the sinus node. An excellent linear relationship has been demonstrated by LOCKHANDWALA (24) between decreases in blood pressure and increases in heart rate for histamine. Cardiac autonomic blockade cancels out positive chronotropic responses to histamine but not hypotensive responses (24). It is reasonable to conclude that the antagonism of the tachycardia produced by histamine in the presence of histamine receptor blockers reflects an attenuation of the reflex autonomic discharge due to the H_1-antagonist (16) or, simply, to the smaller hypotensive response.

With respect to the effect of histamine administration on renal circulation we found dose-dependent increases in renal arterial flow and decreases in renal vascular resistance although the pattern of the response after histamine i.v. bolus is complex (fig. 2) (7).

Experiments with selective antagonists support the conclusion that both types of histamine receptors participate in the renal vasodilatation elicited by this drug. However H_1-receptors appear to predominate since its blockade produced a significant and important rightward shift of the control dose-response curve. BELL *et al.* (7) also observed substantial reductions in the dilator responses to histamine after mepyramine but no investigation was made with selective H_2-antagonists or combined pretreatment with H_1- plus H_2-receptor blockers.

JOHNSTON and OWEN (21-23), who studied in anesthetized cats the modifications caused by histamine and its selective agonists and antagonists on regional blood flow and distribution of cardiac output, did not reach clearcut results in the renal area. In their study, 2-(2-aminoethyl-pyridine caused renal vasodilatation whereas 4-methyl histamine did not. Mepyramine alone or the combination of mepyramine plus metiamide — but not metiamide alone — antagonized the decreases in renal vascular resistance produced by a single preselected dose of histamine. Although the above suggests the participation of both types of receptors with a predominant role of the H_1-type, a careful appraisal of these data should take into consideration that the renal vascular response to histamine reflects a complex balance between direct (receptor mediated) vasodilatation and reflex (autonomic and humoral) induced vasoconstriction, and a meaningful comparison of the selective agonists would require similar falls in blood pressure which is not the case in the experiments of JOHNSTON and OWEN (21-23).

In consequence, further research *in vivo* under controlled conditions is still needed in order to precisely characterize the relative contribution of H_1- and H_2-receptor stimulation to the renal vascular response to histamine.

In the isolated canine renal artery, histamine (10^{-5} to 10^{-3} M) produced a dose-related decrease in the potassium (67 mM) induced tone. It is of interest to point out the difference between the administered doses of histamine *in vivo* (in the order of 10^{-6} M) and histamine concentrations *in vitro*. Both correlate well with those reported in other studies (14, 24). Important differences may occur between *in vitro and* in *vivo* actions of a drug. In the latter case the drug action may be affected by nervous reflexes, endogenous, hormones and other chemical substances which regulate the tone of the vascular smooth muscle. This seems to be the case of histamine (36). The difference between *in vivo* and *in vitro* concentrations observed in this study warrants further investigation.

The results obtained in the isolated canine renal artery indicated the presence of histaminergic receptors whose activation produced vasodilatation. Previous studies from this laboratory using several

isolated preparations demonstrated that dose-response curves to H_1-mediated histamine effects follow a second-order kinetics (slope of the Hill plot equals two) whereas the H_2-mediated responses follow the usual first-order kinetics (35). Territories with a mixed population of histaminergic receptors gave Hill coefficients between 1 and 2 (6). In the present study the Hill coefficient was calculated (5) to be 1.74 suggesting, the existence of both H_1- and H_2-receptors in the canine renal artery, and the prevalence of the H_1-subtype. Further research using selective agonists and antagonists is needed to confirm these preliminary results.

A physiological role for histamine in regulating renal blood flow as suggested in systemic circulation (12, 13, 37, 38) seems unlikely giving the relative high concentrations needed to obtain responses. Such a physiological role has also been denied in other systemic (42) and regional (20, 21-23) circulations.

In conclusion, the present study indicates the existence of two classes of histaminergic receptors in the renal artery of the dog, both mediating dilator responses, although the H_2-subtype appears to predominate.

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Resumen

Se estudia la modificación, tras pretratamiento con antagonistas selectivos H_1 y H_2, de la curva dosis-respuesta (CDR) a la histamina para la frecuencia cardíaca, presión arterial, flujo arterial renal y resistencia vascular renal en perros anestesiados, y las características de la CDR a la histamina en arteria renal aislada de perro. *In vivo*, el pretratamiento con metiamida (10 mg/kg i.v.) no modifica la CDR a la histamina. En contraste, se observa un desplazamiento significativo hacia la derecha de la CDR a la histamina para todos los parámetros hemodinámicos tras tratamiento con difenhidramina (5 mg/kg i.v.). El pretratamiento combinado con metiamida y difenhidramina produce desplazamientos adicionales hacia la derecha de la CDR a la histamina. El análisis de las CDR para el efecto relajante de la histamina en arteria renal aislada de perro previamente despolarizada (K^+ 67 mM) permite obtener una DE50 de $3{,}3 \times 10^{-4}$ M y un coeficiente de Hill de 1,74. Se demuestra que los dos tipos de receptores histaminérgicos existen en la arteria renal del perro mediando respuestas vasodilatadoras, aunque el subtipo H_1 parece predominar en la producción de dicha respuesta.

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