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GUANETHIDINE-INDUCED VASODILATATION IN THE RABBIT, MEDIATED BY ENDOGENOUS HISTAMINE

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1 The effects of guanethidine (0.5–4 mg/kg i.v.) on arterial pressure, hindlimb blood flow and hindlimb vascular resistance (HVR) were studied in unanaesthetized rabbits subjected to 'total' autonomic block.

2 Evidence that this response was mediated by histamine release was that (a) ^{3}H-labelled histamine levels in the hindlimb venous blood rose substantially after guanethidine; (b) infusion of exogenous histamine caused an inhibition of the guanethidine-induced vasodilatation; and (c) competitive antagonism of the response was obtained with the H_2-antagonist burimamide.

3 There was good correlation between the [^{3}H]-histamine release and the time course of the vasodilator response. Glyceryl trinitrate infusions that lowered HVR substantially, did not cause release of histamine.

4 Reserpine, desipramine and indomethacin pretreatment did not alter the vasodilator response to guanethidine.

5 The guanethidine vasodilator response was not influenced by the H_1-antagonist mepyramine or by the other H_2-antagonists, metiamide or cimetidine. The vascular receptors stimulated by endogenous histamine may be distinctive from those stimulated by exogenous histamine, or the action of guanethidine may involve greater production of histamine at an intracellular site that is more readily reached by burimamide than by the other H_2-antagonists.

Introduction

In the course of other studies in the rabbit we observed that the intravenous injection of guanethidine elicited a prolonged vasodilatation (White, 1967; West, Angus & Korner, 1975). Such a response has been reported previously in the rabbit (Kadzielawa, 1962). A preliminary investigation suggested that the dilatation was not the result of autonomic blockade. MacIntosh & Paton (1949) showed that organic bases containing a guanidine group are powerful histamine releasing agents. In view of the recent demonstration by LeBlanc, Côté, Dore & Rousseau (1972) that guanethidine caused a marked elevation in histamine secretion in male rats it seemed possible that its vasodilator action in the rabbit might be mediated through the release of histamine.

In the present study we have examined the effects of histamine antagonists on the vascular response of the hindlimb to guanethidine in autonomically blocked unanaesthetized rabbits (to avoid compensatory reflexes and catecholamine release). Further, we have examined the release of isotopically labelled histamine from the venous effluent. Taken in conjunction with our previous analysis of the effects of histamine bolus injections and infusions (Angus, Bobik & Korner, 1977) these studies suggest that the hindlimb vasodilator response of the rabbit is mediated through the release of histamine, but that the receptor involved shows certain differences from the vascular H_2-receptors responding to intravenously injected histamine.

Methods

Animals and operations

Male New Zealand white rabbits weighing 2.4 to 3 kg were used in these experiments. A preliminary operation was performed under open circuit halothane (ICI) anaesthesia after induction with propranidid (Bayer) 30 mg/kg intravenously. A Doppler ultrasonic cuff type blood flow transducer (4 mm i.d.) and silastic balloon were placed around the lower abdominal

aorta just above the iliac bifurcation with the wires and balloon catheter buried subcutaneously.

On the day of the experiment at least two weeks after the above operation, the central ear artery and vein were cannulated under 0.5% lignocaine local anaesthesia (Korner, 1965). Arterial pressure, heart rate, lower aortic blood flow (taken to be hindlimb blood flow, HBF) and hindlimb vascular resistance (HVR, computed by an analogue divider as arterial pressure/HBF) were determined as described previously (White, McRitchie & Porges, 1974; West *et al.*, 1975). As used, the flow transducer becomes firmly adherent to the artery and the calibration lines are independent of perfusion pressure between the limits tested in these experiments of 60 to 160 mmHg. Changes in vessel calibre following administration of guanethidine and other drugs were assessed from alterations in HVR. It should be noted that in the presence of large arterial pressure changes the resistance changes may be due to myogenic effects secondary to changes in wall tension as well as to the direct effects of the drug used (Folkow, 1964).

Experimental protocol

The experiment began 30 min after completion of the minor procedures. After an initial series of haemodynamic observations all animals were subjected to 'total' autonomic blockade (see Drugs). This was well tolerated and the experiments began 90 min after initiation of 'total' autonomic block. Guanethidine injections 0.5 to 4.0 mg/kg were given intravenously in a random order allowing at least 5 min recovery at control levels between successive doses. Histamine was infused intravenously at rates of 10 to 40 $\mu g\ kg^{-1}\ min^{-1}$ by means of a Harvard slow infusion pump. After a 10 min control period, the infusion was started at the lowest rate for at least 6 min (or until the circulation had stabilized) before doubling the infusion rate. The H_1-antagonist mepyramine maleate (0.8 mg/kg i.v.) was injected every hour and was adequate to block completely the constrictor effect of a 40 μg/kg histamine bolus (Angus *et al.*, 1977). The H_2-antagonists burimamide and metiamide were both made up in 0.9% w/v NaCl solution (saline) after first dissolving the drugs in 1.0 N HCl and then buffering the solution with 0.1 N NaOH as recommended by Smith, Kline & French Laboratories. Infusions of either burimamide 0.35 mg $kg^{-1}\ min^{-1}$ or metiamide 0.4 mg $kg^{-1}\ min^{-1}$ (i.e. both 1.65 $\mu mol\ kg^{-1}\ min^{-1}$) were given for at least 10 min before repeating the histamine or guanethidine dose-response experiments. The infusion of the H_2-antagonist was continued for the remainder of the experiment. These H_2-antagonist infusion rates were adequate to block the dilator response of both histamine bolus and infusions of 40 $\mu g\ kg^{-1}\ min^{-1}$ after mepyramine (Angus *et al.*, 1977). Other experiments involving several other drugs are discussed in Results.

Experiments with [^{3}H]-histamine

Preparation. Rabbits were prepared with a Doppler flow transducer as described above, and after total autonomic block, a continuous infusion of ^{3}H-labelled histamine (1.5 μCi/min) was given into the thoracic aorta so as to allow the [^{3}H]-histamine in the hindlimb arterial and venous blood to reach an equilibrium with vascular histamine stores and metabolism. Cross (1973) has shown that the blood levels of labelled histamine declined extremely rapidly after intravenous bolus injection in rats. Samples (2 ml) of inferior vena caval blood were withdrawn (through a small catheter) over 30 s and frozen. Three control samples were taken at 5 min intervals before giving either guanethidine (4 mg/kg) or compound 48/80 (1 mg/kg). Samples were taken periodically for the next 20 minutes. In a second series of 5 rabbits the effect of a lower dose of guanethidine (2 mg/kg) was compared with an infusion of glyceryl trinitrate (GTN) on the release of [^{3}H]-histamine from the hindlimb. GTN infusion rates were chosen to lower HVR to similar values as those produced by guanethidine, to test whether vasodilation *per se* influenced [^{3}H]-histamine content in venous blood.

Analysis. [^{3}]-histamine was extracted from haemolysed blood by homogenization with 0.4 N perchloric acid in a ratio of 1:5. Control experiments showed this procedure to extract 91.5 $\pm$ 3.8% (mean $\pm$ s.d.) of [^{3}H]-histamine. After centrifugation of the homogenate to remove precipitated protein, [^{3}H]-histamine in the perchloric acid extract was determined by the reverse isotope dilution method of Schayer (1971) with the following modifications. After derivatization of extracted [^{3}H]-histamine and [^{3}H]-methylhistamine with benzenesulphonyl chloride, the resultant benzenesulphonyl derivatives ([^{3}H]-BSH and [^{3}H]-BSMH, see Drugs) were extracted from the reactant mixture with chloroform which was washed with water and evaporated to dryness. The residue was dissolved in acetone and chromatographed on silica gel plates in methanol:chloroform (5:95). This procedure separates BSMH (R_F 0.28) and BSH (R_F 0.83). The BSH fraction was eluted from the silica gel with acetone and after evaporation of the acetone in a stream of nitrogen the specific activity of [^{3}H]-BSH was determined. Subsequent recrystallization of [^{3}H]-BSH from aqueous acetone showed its specific activity after the chromatographic procedure to be constant. All results have been corrected for recovery of [^{3}H]-histamine from the perchloric acid extract.

Rat mast cell preparation

A rat peritoneal mast cell suspension was made according to the method of Johnson & Moran (1966). Duplicate 0.5 ml aliquots of cell suspension were preincubated for 15 min at 37°C with either 0.25 ml metiamide or burimamide (0.6 mM and 0.7 mM respectively, final concentration) or a saline control. A range of guanethidine sulphate concentrations giving final values of 0, 0.03, 0.34, 1.69, 3.37 mM was added. After 5 min the tubes were centrifuged for 5 min and 0.75 ml of supernatant was added to 1.25 ml distilled water and 0.5 ml trichloroacetic acid. After further centrifugation 2 ml of supernatant was assayed fluorometrically for histamine (Kremzner & Wilson, 1961). The results are expressed as percentage of the maximum histamine released as determined by boiling the mast cell suspension. All drugs were dissolved in saline and the reaction adjusted to pH 7.0. Control experiments were carried out to ensure that the histamine assay was not affected by any of the other drugs or drug combinations used in this study.

Total autonomic blockade

The drug regime to block the autonomic effectors was as described by Korner & Uther (1969), with the modifications described by West *et al.* (1975). A large dose of guanethidine sulphate (12.5 mg/kg i.v.) was injected over 1 min; to help rapid restoration of blood pressure the animals were given 20–30 ml of 5% dextran solution 15–20 min after starting the injection of guanethidine. They also received atropine sulphate, an initial intravenous dose of 1 mg/kg followed by a continuous infusion of 0.1 mg kg^{-1} min^{-1} throughout the experiment to block vagal effectors (Korner, Uther & White, 1968) and intravenous propranolol hydrochloride 0.5 mg/kg followed by an infusion of 0.04 mg kg^{-1} min^{-1}. In addition they received an intravenous injection of phentolamine mesylate 2 mg/kg followed by 1 mg/kg every 40 minutes.

Lumbar sympathetic nerve stimulation experiments in anaesthetized rabbits show that the above dose of guanethidine blocks transmission in sympathetic nerves to strong electrical stimuli (6.5 V, 1 ms, 8 Hz) for a period of 4 to 6 h (West *et al.*, 1975). Guanethidine does not interfere with secretion of adrenal catecholamines but the doses of propranolol and phentolamine were adequate to block heart rate and peripheral β- and α-adrenoceptor mediated effects produced by 4 μg of isoprenaline sulphate and by 2 μg/kg noradrenaline injected intravenously.

Drugs

The following drugs were used: guanethidine sulphate (Ciba-Geigy), histamine acid phosphate (Sigma), phentolamine mesylate (Ciba-Geigy), propranolol hydrochloride (ICI), mepyramine maleate (May & Baker), burimamide, metiamide and cimetidine (Smith, Kline & French), compound 48/80 (Sigma), papaverine (Knoll Labs), glyceryl trinitrate (Burroughs Wellcome), reserpine (Ciba-Geigy), indomethacin (Merck, Sharp & Dohme), desipramine hydrochloride (Ciba-Geigy), bretylium tosylate (Burroughs Wellcome), debrisoquine sulphate (Roche), bethanidine sulphate (Burroughs Wellcome) guanoxan sulphate (Pfizer), N-(β-guonidinoethyl)-hexahydrobenzo-(+)-azocine sulphate (Ph 881/1-Pharmacia), N-(2-(hexahydro-1-azepinyl)ethyl) acetamidine hydrochloride (BW 1113C60—Burroughs Wellcome), 3,5-dimethyl- 4-(2-ethoxy-1-trimethylammonium) benzophenone bromide (BW 172C58—Burroughs Wellcome), histamine-[2,5-^{3}H] dihydrochloride (Amersham), 1-methylhistamine dihydrochloride (Calbiochem). Di-benzene sulphonyl histamine (BSH) and benzene sulphonyl-1-methylhistamine (BSMH) were synthesized according to Schayer (1971). Structures of BSH & BSMH were verified by mass spectrometry. Silica gel used was kieselgel GF 254.

Calculations

HVR changes following histamine infusions were calculated as percentage of control HVR (100%) taken immediately before the start of infusion. Peak fall in HVR following a guanethidine injection was expressed as percentage fall from resting HVR just before injection. Mean values, standard errors of the mean (s.e. mean) and regression analysis were calculated where required (Snedecor & Cochran, 1969). For the [^{3}H]-histamine assay results, each sample was expressed as a percentage of the control counts (mean value from the control determinations was taken as 100%). In calculating the counts and flow product, the counts were multiplied by the Doppler flow (kHz) measured simultaneously with the collection of the venous sample for the [^{3}H]-histamine assay. The results were calculated as percentage of the control product as for counts alone.

Results

Nature of vasodilator response to guanethidine

The method of producing 'total' autonomic block in the standard preparation in this study consisted of initial administration of 12.5 mg/kg guanethidine plus the other blocking agents described in the Methods section. Administration of guanethidine resulted in pronounced reduction in blood pressure and HVR (Figure 1, left panels). When the guanethidine injection was repeated 1 h later in these autonomically

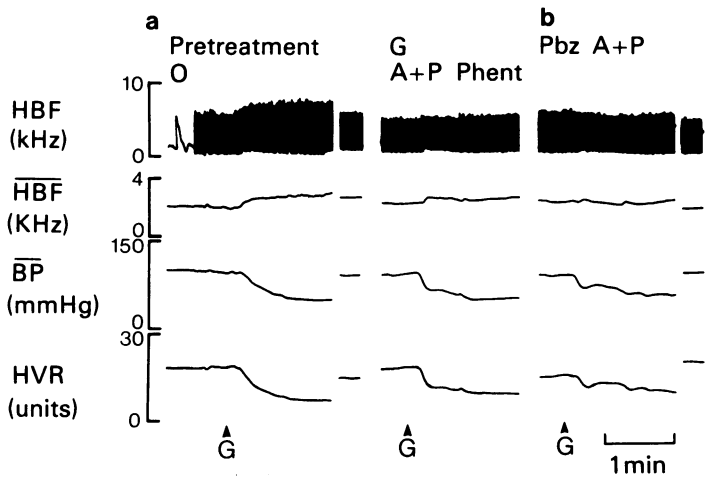

Figure 1 Haemodynamic changes following intravenous guanethidine (G) 12.5 mg/kg (at arrowhead). *From top:* phasic and mean hindlimb blood flow (HBF, kHz), mean blood pressure (BP, mmHg), mean hindlimb (lower aorta) vascular resistance (HVR, units). (a) and (b) are experiments performed on different days. (a) *Left panels*; administration of guanethidine in the absence of pretreatment with other drugs; note that variables had returned towards control after 60 min (next panel). At the second arrowhead guanethidine was given in the presence of 'total' autonomic block produced by pretreatment with guanethidine (G); atropine + propranolol (A + P); phentolamine (Phent). (b) Administration of guanethidine in the presence of 'total' autonomic block produced by pretreatment with phenoxybenzamine (Pbz); atropine + propranolol (A + P), administered to the same rabbit as (a) one week later. See methods section for doses.

blocked animals, the haemodynamic effects were similar to those observed after the first injection (Figure 1, middle) despite good evidence of effective neural block at this time (West *et al.*, 1975). When the sympathetic vasoconstriction was initially blocked with phenoxybenzamine (6 mg/kg) instead of guanethidine (12.5 mg/kg), injection of guanethidine again resulted in a marked fall in HVR (Figure 1, right). These results suggest that the most of the vasodilatation following guanethidine is not mediated by the autonomic nervous system.

Histamine antagonists and guanethidine

The hindlimb vasodilator response evoked by guanethidine in the rabbit with 'total' autonomic block was dose-dependent up to 2 mg/kg. Increasing the dose to 4 mg/kg did not significantly increase further the peak fall in HVR (Figure 2). In these 5 rabbits the subsequent infusion of the H_2-receptor antagonist burimamide (0.35 mg kg^{-1} min^{-1}) significantly attenuated the fall in HVR after guanethidine at each dose used ($P < 0.01$ for within animal comparisons at each dose.) There was a 4.6 fold shift calculated from the regression equation and the regression lines were parallel (slopes were 48 $\pm$ 10.6 and 52 $\pm$ 5.6, mean $\pm$s.e. mean, for control and burimamide lines respectively) suggesting that burimamide was acting competitively in reducing the vasodilator

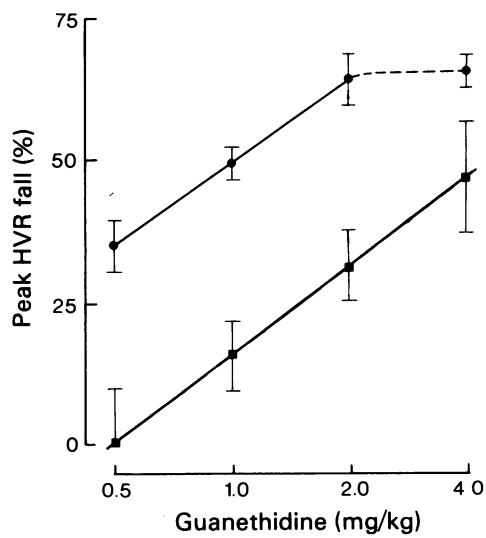

Figure 2 Linear regression lines (vertical lines show s.e. means) relating the peak fall in hindlimb vascular resistance (HVR) (as % of resting HVR) to the dose of guanethidine in five rabbits. (●) No histamine antagonists; (■) responses during burimamide infusion (0.35 mg kg^{-1} min^{-1}). Dotted line on control line joins the 2 and 4 mg/kg HVR responses since the latter was not used in the regression calculation and the point represents the mean $\pm$s.e. mean HVR values.

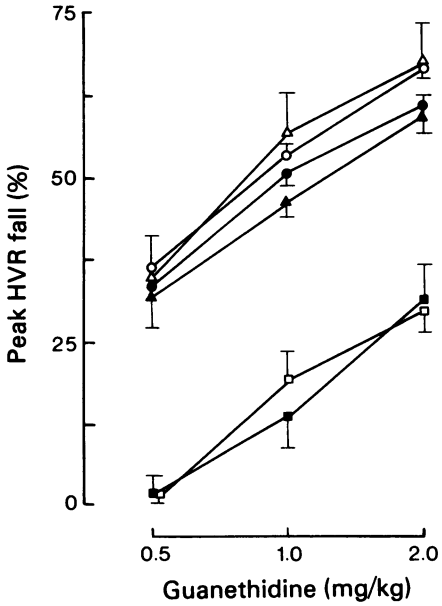

Figure 3 The effect of histamine antagonists on the dose-dependent fall in hindlimb vascular resistance (HVR) after guanethidine. Points represent mean peak HVR fall (as % of resting HVR), vertical lines show s.e. mean). Different histamine antagonists as follows:— (●) no histamine antagonist ($n = 15$); (▲) metiamide, 0.4 mg kg^{-1} min^{-1} ($n = 6$); (○) mepyramine 0.8 mg/kg ($n = 10$); (■) burimamide 0.35 mg kg^{-1} min^{-1} ($n = 5$); (△) metiamide 0.4 mg kg^{-1} min^{-1} and mepyramine 0.8 mg/kg ($n = 4$); (□) burimamide 0.35 mg kg^{-1} min^{-1} and mepyramine 0.8 mg/kg ($n = 6$); (n = number of experiments).

response to guanethidine. Even in the presence of burimamide no constrictor response to guanethidine was ever observed. In subsequent experiments pretreatment with mepyramine (0.8 mg/kg) alone (10 rabbits) had no significant effect on the guanethidine dose-related reduction in HVR (Figure 3). In 6 of these rabbits additional administration of burimamide (0.35 mg/kg^{-1} min^{-1}) produced a marked attenuation of the dilator response ($P < 0.01$ at all doses) with a similar shift in the curve as observed with burimamide alone (Figure 3). However, in the remaining 4 rabbits pretreated with mepyramine, metiamide infusion at the same mol dose rate as for burimamide failed to alter the dilator response to guanethidine. Six additional rabbits were given metiamide (0.4 mg kg^{-1} min^{-1}) alone in the absence of mepyramine but again this did not have any effect on the magnitude of the guanethidine-induced vasolilatation. In one experiment the metiamide infusion rate was increased 10 times (i.e. 4 mg kg^{-1} min^{-1}) for 1 h but this still failed to influence the guanethidine vasodilatation.

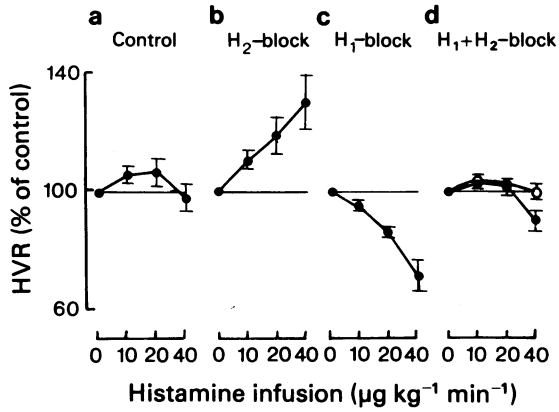

Figure 4 Mean hindlinb vascular resistance (HVR) (percent of resting) during infusions of histamine at different rates (μg kg^{-1} min^{-1}). Vertical lines show s.e. mean. (a) Control, no histamine antagonists: $n = 12$; (b) H_2-block, pretreatment with metiamide, 0.4 mg kg^{-1} min^{-1} $n = 4$; (c) H_1-block, pretreatment with mepyramine, 0.8 mg/kg $n = 8$; (d) H_1-and H_2-block, pretreatment with mepyramine 0.8 mg/kg and burimamide, 0.35 mg kg^{-1} min^{-1} $n = 4$, (■) pretreatment with mepyramine 0.8 mg/kg and metiamide, 0.4 mg kg^{-1} min^{-1} $n = 4$ (○).

Cimetidine infusion (1.6 μmol kg^{-1} min^{-1}) was also without effect in 2 rabbits.

Histamine infusions (10 to 40 μg kg^{-1} min^{-1}) in the same series of animals had little effect on HVR (Figure 4) in agreement with previous findings (Angus *et al.*, 1977). This was because in the rabbit, the H_1-mediated constrictor response virtually masks the H_2-mediated vasodilatation so that the response from each receptor type only becomes apparent during administration of the appropriate antagonist (Figure 4). It should be noted that the H_2-mediated vasodilatation produced by histamine infusions was blocked equally by burimamide or metiamide.

Effect of histamine antagonists on other vasodilators

Mepyramine, burimamide and metiamide (in effective anti-histamine doses) were all without effect on the vasodilator response (fall in HVR to about 50% of resting) produced by adenosine 250 μg kg^{-1} min^{-1} and by papaverine (0.5–2 mg/kg).

Interaction between guanethidine and exogenous histamine

Since the vasodilator response to guanethidine was blocked by burimamide, this raised the possibility that it might be mediated through a histamine receptor. Therefore we examined the possibility of an interaction between simultaneous administration of hista-

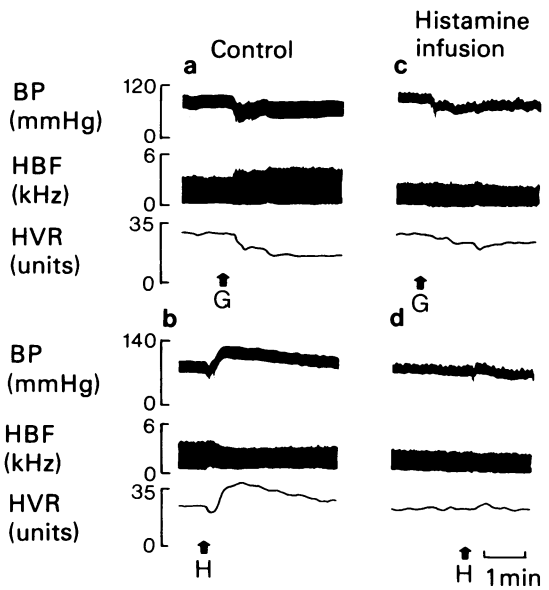

Figure 5 Haemodynamic changes following intravenous guanethidine (G) 2 mg/kg and intravenous bolus histamine injections (H) 40 μg/kg in the same rabbit. From top: phasic blood pressure (BP), phasic hindlimb blood flow (HBF), and mean hindlimb vascular resistance (HVR); (a) control response to administration of guanethidine and (b) bolus histamine injection; (c) guanethidine and (d) histamine responses during the continuous infusion of histamine, 200 μg kg^{-1} min^{-1}.

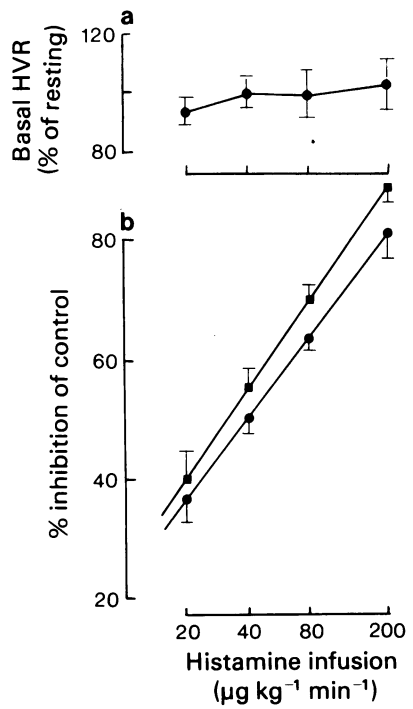

Figure 6 (a) Basal hindlimb vascular resistance values (HVR) (mean in 4 rabbits) during histamine infusion at rates between 20 and 200 μg kg^{-1}. min^{-1}. HVR values are expressed as % of resting HVR (100%) before the histamine infusion started. Vertical lines show s.e. mean. (b) Linear regression lines of percent inhibition of dilator response (control + 100%) to guanethidine 2 mg/kg (●), and constrictor response to bolus histamine injection 40 μg/kg (■) during histamine infusion at rates between 20 and 200 μg kg^{-1} min^{-1}. Vertical lines show s.e. mean at each infusion rate.

mine and guanethidine. In 4 rabbits control responses were obtained for a standard dose of guanethidine (2 mg/kg) (Figure 5a) and the injections were then repeated during histamine infusions at each of the following rates: 20, 40, 80 and 200 μg kg^{-1} min^{-1} when the haemodynamics were stable (Figure 5c). Over the entire range of histamine infusions there was balance between the H_1-mediated constrictor effects and H_2-mediated dilator effects (cf. Figure 4) and the HVR preceding each guanethidine injection did not alter over the entire range of histamine infusions (Figure 6a). With rising rates of histamine infusion the peak reduction in HVR after each guanethidine injection became progressively smaller until the response was inhibited by 80% at the highest infusion rate (Figure 6b).

We also analysed the effects of the exogenous histamine infusions on the magnitude of the H_1-mediated vasoconstrictor response obtained after bolus injection of histamine. Under control conditions before histamine infusion bolus injection caused a biphasic short lived fall in HVR followed by a larger and more prolonged rise (Figure 5b). Angus *et al.* (1977) have shown that the former is mediated through H_2-receptors while the latter is mediated through H_1-receptors. Moreover after bolus injections the H_1-constrictor effects are more prominent than the H_2-dilator effects contrasting with the balanced effects under the equilibrium conditions of histamine infusions (Angus *et al.* 1977). When a standard bolus dose of histamine (40 μg/kg) was administered during different rates of histamine infusion the H_1-mediated constrictor effect became progressively smaller (Figure 5b, d, Figure 6b). The inhibition of the H_1-constrictor response following histamine bolus was approximately the same as the inhibition of the guanethidine vasodilatation, with the slopes of the regression lines in Figure 6b respectively 48.9 ± 14 (s.e. mean) for the former and 44 ± 7.8 for the latter effect.

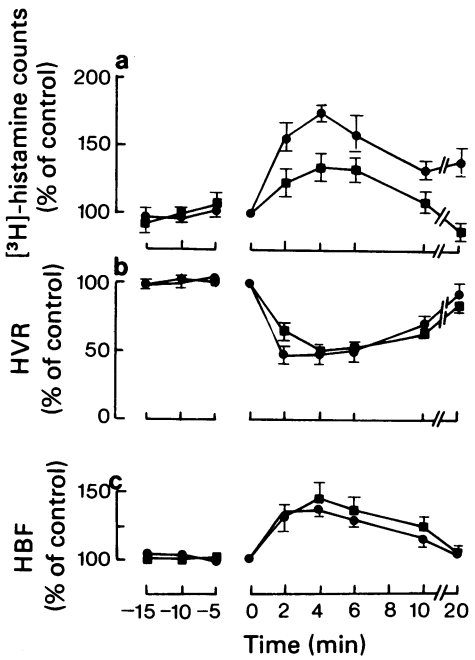

Figure 7 Effect of two doses of guanethidine on histamine release and haemodynamics in the unanaesthetized autonomically blocked rabbit.(a) [^{3}H]-histamine in the inferior vena caval blood; (b) hindlimb vascular resistance (HVR); (c) hindlimb blood flow (HBF). All parameters are expressed as percent of the mean (=100%) calculated from 3 control values taken over 15 min before injecting guanethidine at time 0. Symbols represent two separate groups of rabbits: (●) guanethidine 4 mg/kg (n = 5); (■) guanethidine 2 mg/kg (n = 4). Vertical lines show s.e. mean.

Analysis of the [^{3}H]-histamine release

[^{3}H]-histamine, infused into the thoracic aorta, produced a stable equilibrium in that the [^{3}H]-histamine sampled from the hindlimb venous blood did not alter significantly during the 15 min control period before any injection. In the first series of rabbits, guanethidine (4 mg/kg) caused a significant increase in [^{3}H]-histamine levels in the venous blood. Peak counts (175 ± 7% of control) occurred at 4 min and declined to 135% at 10 and 20 min samples after injection (Figure 7a). The amount of histamine released appeared to be dose-related since half the guanethidine dose resulted in almost half the release of [^{3}H]-histamine (peak counts 134.3 ± 11.1%). The time course for each dose followed the change in HVR but there was no difference in the peak fall in HVR between the two doses (Figure 7b; cf. Figure 2). Since the counts of [^{3}H]-histamine were determined in 1 g of blood collected over 30 s an increase in blood flow may dilute the [^{3}H]-histamine released from the hindlimb, assuming that the arterial concentration remains constant. When this analysis was done (counts × hindlimb flow) the peak increases in counts were 193 ± 15 and 235 ± 11% of control (=100%) for the 2 and 4 mg/kg guanethidine doses respectively.

Compound 48/80

In the first series of experiments 48/80 (1 mg/kg) was administered 1 h after recovery from the guanethidine injection. Two rabbits died with apparent respiratory distress within 6 min of the injection while the other 3 survived at least to 30 minutes. In these 3 rabbits [^{3}H]-histamine counts did not change significantly from control following 48/80. Four minutes after injection of 48/80, [^{3}H]-histamine was 100 ± 17.1% and HVR was 90 ± 10% of control (=100%). There was a small rise in HVR immediately following administration of 48/80 and HVR then fell to about 70% after 10 minutes. Higher doses of 48/80 resulted in death within 6 min in all rabbits. Compound 48/80 was therefore an unsuitable drug to test endogenous histamine release in this species. In one rabbit compound 48/80 was injected chronically (total of 5 mg/kg over 48 h in divided doses) in an attempt to deplete histamine stores. After autonomic block the HVR dose-response relationship for guanethidine was unchanged from a previous experiment in this rabbit.

Glyceryl trinitrate

We used GTN infusions to test whether vasodilatation *per se* was responsible for the increase in [^{3}H]-histamine observed. In 5 experiments GTN (30–100 $\mu g\ kg^{-1}\ min^{-1}$) was infused to lower the HVR to 50% of resting, as was obtained at the peak response to guanethidine. There was no significant change in [^{3}H]-histamine counts. Vasodilatation *per se* was thus not associated with release of [^{3}H]-histamine in the hindlimb.

Action of guanethidine on peritoneal mast cells

Guanethidine caused a dose-dependent release of histamine from peritoneal mast cell preparations (Figure 8). Significant release was caused by relatively low concentrations of drug. Preincubation of the mast cells with either burimamide (0.7 mM) or metiamide (0.6 mM) had no effect on the amount of histamine released by guanethidine.

Reserpine pretreatment

Guanethidine dose-response experiments were carried out in 2 rabbits before and after pretreatment with

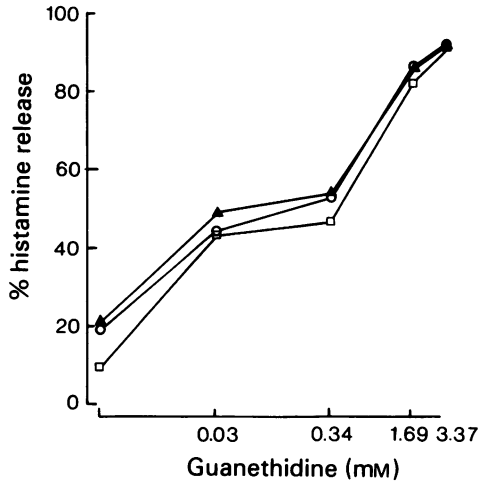

Figure 8 Histamine release (% of maximum) following addition of different doses of guanethidine (mM final concentration) in rat peritoneal mast cell preparation under control conditions (preincubated with saline, ○); after preincubation with burimamide (0.7 mM, ▲); after preincubation with metiamide (0.6 mM, □).

reserpine (2 mg/kg i.p.) for 2 days. There were no significant changes in peak HVR responses to doses of guanethidine from 0.5 to 4.0 mg/kg after reserpine pretreatment.

Indomethacin

To test whether prostaglandins might contribute to the vasodilator response to guanethidine, peak HVR responses to guanethidine (0.5 to 4 mg/kg) were compared before and during prostaglandin synthesis inhibition by indomethacin (9 mg/kg bolus and 1 mg kg^{-1} h^{-1}) in 2 rabbits. No significant reduction in the peak dilator response to any dose of guanethidine was observed after indomethacin.

Desmethylimipramine

Desmethylimipramine (DMI) has been shown to block some of the pharmacological effects of guanethidine including the initial adrenergically mediated rise in pressure in the cat and dog, probably by preventing guanethidine uptake into noradrenergic nerve terminals (Kaumann, Basso & Aramendia, 1965). After obtaining dose-response curves to guanethidine, DMI 3 mg/kg was administered. This dose has been found adequate to abolish completely the rise in pressure and heart rate produced by tyramine and guanethidine in dogs. In our hands the vasodilator action of guanethidine was unaffected in the rabbit by DMI as was the transient dilator response in the dog, suggesting that in both species blockade of adrenergic neuronal uptake was of no consequence to the dilator action of guanethidine.

Other adrenergic neurone antagonists

We tested a number of drugs that are potent adrenergic neurone antagonists (see Boura & Green, 1965) in an attempt to find another 'guanethidine-like' vasodilator in the 'totally' autonomically blocked rabbit. In doses up to 10 mg/kg bretylium tosylate, compound BW 172C58, bethanidine and debrisoquine were either slightly vasoconstrictor or without effect

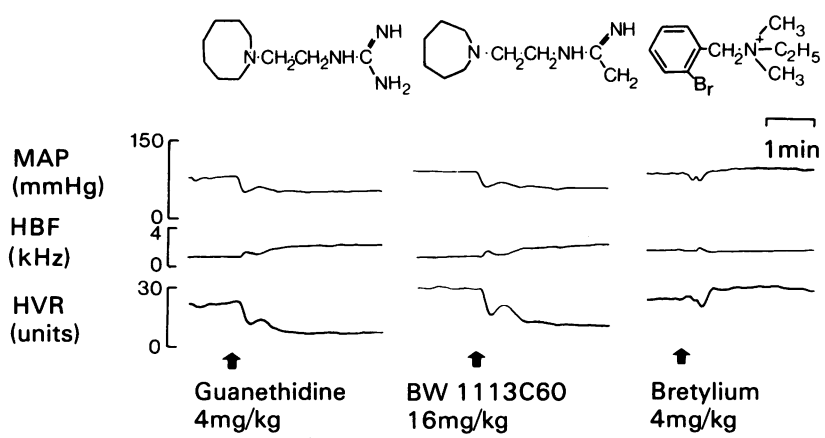

Figure 9 Haemodynamic changes following the administration of 3 different adrenergic neurone blocking drugs in the same rabbit. Parameters are: mean arterial pressure (MAP, mmHg), mean hindlimb blood flow (HBF, kHz) and mean hindlimb vascular resistance (HVR, units). At top are shown molecular structure of the 3 drugs.

on HVR. Guanoxan did cause some transient vasodilatation. Compound Ph 881/7 (mol. wt. 295) was toxic at 4 mg/kg and caused death in doses greater than 8 mg/kg. However, compound BW 1113C60 (mol. wt. 219) caused dose-related vasodilatation with a time-course of action similar to guanethidine (Figure 9). In 3 rabbits the dilator potency of BW 1113C60 was approximately 0.25 that of guanethidine (mol. wt. 247) calculated from the peak fall in HVR over a range of 0.5 to 16 mg/kg for BW 1113C60.

Discussion

The dose-related vasodilator response to guanethidine in the rabbit hindlimb is not, for the most part, mediated through the autonomic effector pathways. The fall in HVR could be somewhat exaggerated by a myogenic effect on wall tension, secondary to the large reduction in transmural pressure (Folkow, 1964). Johnson (1968) has found that myogenic arteriolar dilatation in the cat mesentery is maximal immediately after reducing transmural pressure but that after 0.5 to 1 min the residual effect is small. In addition, West *et al.* (1975) have found that in normotensive rabbits the fall in HVR is only slightly exaggerated when transmural pressure is reduced after autonomic block. In the present study the guanethidine-induced vasodilatation is dependent neither on reserpine-sensitive tissue catecholamine stores nor on the noradrenergic neuronal uptake mechanism. Furthermore the response was not altered after administration of the prostaglandin synthetase inhibitor, indomethacin.

In the autonomically blocked rabbit the following points favour the view that the guanethidine-mediated vasodilatation is mediated through the release of endogenous histamine:— (1) increased release of labelled histamine by the drug; (2) inhibition of the guanethidine vasodilator response during infusions of histamine; (3) competitive antagonism by the H_2-antagonist burimamide. The studies of MacIntosh & Paton (1949) have shown that basic molecules containing a guanidine group are powerful histamine releasing agents. An alternative possibility, that gunaethidine has a direct effect on histamine receptors is unlikely from consideration of its chemical structure (Barlow, 1964), and from the absence of effect of the drug on the isolated ileum and the adrenal pouch preparation (Mongar & Schild, 1952; Paton, 1957; Athos, McHugh, Fineberg & Hilton, 1962).

The release of [^{3}H]-histamine in the present experiments is dose-related. In the assay experiments the two doses used were at the upper end of the physiological dose-response curve so that the maximum dilator effects were similar. However, there is good correlation between the [^{3}H]-histamine release and the time-course of the vasodilator response. The increase in [^{3}H]-histamine release following administration of guanethidine does not necessarily mean that the histamine comes from the same tissue sites at which guanethidine acts. It could be merely coincidentally released but in that case it is not the result of vasodilatation *per se*, in view of the absence of histamine releases with glyceryl trinitrate. Due to the rapid metabolism of histamine we were unable to produce a model of endogenously synthesized [^{3}H]-histamine by infusing labelled histidine. However, the effects of guanethidine in the rat mast cell preparation have shown that guanethidine does indeed release histamine at least from one type of cell. The particular model probably has no direct relevance to the rabbit where mast cells are sparse (Riley, 1959 and personal observations). The latter is in accord with the absence of increase in [^{3}H]-histamine by compound 48/80 in the rabbit, which is in agreement with the results of Schachter (1953) and of Papacostas & Loew (1959). Histamine release from rat mast cells is unaffected by burimamide, but we have not investigated in the present study whether burimamide alters [^{3}H]-histamine release from the hindlimb.

The possible competitive antagonism between the H_2-antagonist burimamide and the vasodilator action of guanethidine is also consistent with an effect by the latter on vascular histamine receptors. In the present study we used only a single dose of burimamide and therefore cannot say whether other criteria of simple competitive antagonism were fulfilled (Arunlakshana & Schild, 1959). However, the effect of burimamide appears to be specific for histamine and for the guanethidine response, and there has been no antagonism to the vasodilatation produced by adenosine or papaverine in our study or to 5-hydroxytryptamine, adenosine triphosphate, bradykinin and acetylcholine (Lorenz, Thermann, Hamelmann, Schmal, Maroske, Reimann, Kusche, Schingale, Dormann & Keck, 1973). In the cat, burimamide releases adrenal catecholamines (Hood, Smy & Weetman, 1975) and also has α-adrenoceptor blocking properties (Brimblecombe, Duncan, Owen & Parsons, 1976) but such effects have been excluded by the autonomic blocking agents used in the present study.

Burimamide effectively blocks the guanethidine-induced vasodilatation. In contrast, metiamide and cimetidine are completely ineffective in blocking this response even though they are satisfactory antagonists of the H_2-mediated vascular response to exogenous histamine. Other workers have observed differences between the various H_2-receptor antagonists. For example, Brimblecombe *et al.* (1976) have noted that in the rat, burimamide antagonized compound 48/80-induced paw oedema more effectively than metiamide. It is possible that the antagonistic effect

of burimamide on the guanethidine-induced vasodilatation is unrelated to its capacity to block H_2-receptors. However the interaction between infused histamine and the guanethidine vasodilatation and the results of the [^{3}H]-histamine experiments strongly suggests that endogenously released histamine does play a role in the guanethidine response. It thus seems likely that the endogenously released histamine is through an 'H_2-like' vascular receptor with somewhat different steric properties from the H_2-receptor accessible to histamine arriving by the vascular route. Since the guanethidine vasodilatation is not influenced by mepyramine it would appear that any intraluminal effect of released histamine on H_1-receptors becomes masked by stimulation of extraluminal 'H_2-like' receptors. An alternative possibility is that guanethidine acts through some intracellular action involving histamine which is much more accessible to burimamide than to metiamide or cimetidine.

We have not investigated how guanethidine increases endogenous histamine production. The source of histamine could be partly in the vascular wall as suggested recently by El-Ackad & Brody (1975). Either release of histamine from the vascular wall or inhibition of histamine metabolism (Le Blanc *et al.*, 1972) could underlie the greater production of histamine in the present study.

In conclusion, the vasodilator effects of guanethidine in the hindlimb of the autonomically blocked rabbit appear to be mediated through the release or metabolic effects of endogenous histamine. The mechanism of the vasodilatation appears to be different from the H_2-mediated action of exogenous histamine, suggesting that it either occurs through an 'H_2-like' receptor (with properties distinctive from H_2-vascular receptors accessible to infused histamine) or through an intracellular effect.

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