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INFLUENCE OF HISTAMINE H_1- AND H_2-RECEPTOR BLOCKERS ON SYMPATHETIC VASODILATOR AND VASOCONSTRICTOR RESPONSES IN CANINE PAW

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1 Vasodilator responses to histamine, bradykinin and sympathetic nerve stimulation were elicited in the perfused paw of dogs treated with bretylium (15-20 mg/kg) and atropine. The H_2-receptor blocking agent, burimamide, when administered in the dose of 5 mg/kg intravenously and 4 mg intra-arterially did not depress significantly these vasodilator responses. The subsequent administration of tripelennamine in the dose of 2.5-5 mg/kg intravenously and 4 mg intra-arterially produced a significant blockade of the response to histamine and reduced the sustained vasodilator response to nerve stimulation.

2 In guanethidine-treated dogs, tripelennamine administered in the same dose following burimamide produced a blockade of the respone to histamine comparable to that in the bretylium experiments, but decreased only the sustained vasodilator response to stimulation at 1 Hz. When the order of administration of the antihistamines was reversed in another group of guanethidine-treated dogs, tripelennamine had only a slight blocking effect on the response to histamine and did not affect the responses to nerve stimulation. Burimamide exerted a significant blocking effect on the response to histamine, but not to nerve stimulation. Another H_2-receptor blocking agent, metiamide, when given after tripelennamine, also had a marked blocking effect on the response to histamine and almost abolished the vasodilator response to 4-methylhistamine, an H_2-agonist. Nevertheless, even in the presence of this profound histamine blockade, the sustained vasodilator response to nerve stimulation remained unchanged.

3 In another group of experiments vasoconstrictor responses to exogenous noradrenaline and sympathetic stimulation were initially depressed by burimamide and later returned to control values. Tripelennamine increased these responses by its uptake blocking action.

4 It is concluded that the sustained vasodilator response is not antagonized by a specific antihistaminic action. The decrease in the sustained vasodilator response produced by antihistamines is probably attributable to potentiation of a residual adrenergic vasoconstrictor effect not completely blocked by bretylium.

Introduction

The introduction of burimamide (*N*-methyl-*N'*-4(-[4(5)-imidazolyl] butyl thiourea) has permitted an examination of certain actions of histamine which were previously resistant to blockade by antihistamines. This agent when given alone blocks the effects of histamine on gastric acid secretion and on the atrium, and when combined with mepyramine blocks the depressor action of histamine in the cat (Black, Duncan, Durant, Ganellin & Parsons, 1972). It was postulated by Black *et al.* (1972) that burimamide occupies histamine H_2-receptors to induce this blockade. H_2-receptors are believed to mediate vasodilatation and H_1-receptors vasoconstriction in the pulmonary vascular bed of the guinea-pig (Goadby & Phillips, 1973; Turker, 1973). The vasoconstrictor effect of histamine was reversed to a vasodilator response by the H_1-receptor blocking agent, mepyramine, and the vasodilatation was subsequently abolished by the H_2-receptor blocking agent, burimamide.

In previous studies it was reported that a vasodilator response of long duration was evoked by sympathetic nerve stimulation in the dog's paw or hind limb after adrenergic neuronal blockade or reserpine (Zimmerman, 1966; Beck, Pollard, Kayaalp & Weiner, 1966). This response has been designated sustained vasodilatation (Beck *et al.*,

1966). The response is predominantly mediated by a non-cholinergic transmitter since only the initial rapidly developing component is blocked by atropine (Beck *et al.*, 1966; Brody & Schaffer, 1970). Antihistamines reduce, but do not block sustained vasodilatation (Zimmerman, 1966; Rolewicz & Zimmerman, 1972). The inability to block this response may be attributable to the participation of H_2-receptors. If histamine is the mediator of sustained vasodilatation it might be assumed that the combined administration of an H_1- and H_2-blocker would be capable of abolishing the response. The present investigation was planned to determine the degree to which sustained vasodilatation elicited in the dog's paw by sympathetic stimulation is blocked by combined administration of an H_1-blocker, tripelennamine and an H_2-blocker, burimamide or metiamide. Also, since little is known about the effect of antihistamines on the sympathetic adrenergic system, especially with respect to the vasculature, their influence on vasoconstrictor responses to adrenergic nerve stimulation was also examined.

Methods

Dogs of either sex weighing 13.6-30.0 kg were anaesthetized by the intravenous administration of pentobarbitone sodium (30 mg/kg), and supplements of 30 mg were injected when necessary. Skeletal muscle relaxation was induced with decamethonium bromide (0.25 mg/kg), and this was supplemented at 30-60 min intervals with injections of 1 mg of the drug. All animals were artificially ventilated with a Harvard respirator. The left hind paw was prepared for perfusion as described by Zimmerman (1966). Briefly, this consisted of exposing the cranial tibial and lateral saphenous arteries in the paw and the left and right femoral arteries. Heparin sodium (7.5 mg/kg) was injected intravenously to prevent clotting. The paw was perfused by leading blood from the right femoral artery to a T 6S Sigmamotor pump which maintained flow constant through the catheterized cranial tibial artery. The left femoral and lateral saphenous arteries were ligated to isolate the arterial circulation of the paw. Systemic arterial and paw perfusion pressures were recorded on either a Gilson polygraph or Beckman dynograph. Since blood flow was constant, changes in perfusion pressure indicated changes in vascular resistance of the paw. Flow averaged 28.5 ± 0.7 ml/min (±s.e. mean) in the 26 experiments of this study. The left lumbar sympathetic nerve was stimulated with a Grass stimulator using pulses of 15-20 V, 1 ms in duration at 1, 3 and 10 Hz. The period of stimulation was in all cases 30 seconds. Vasodilator agonists were administered intra-arterially to the paw by bolus injections and antihistamines by slow infusions, intra-arterially and intravenously.

Sustained vasodilatation

At the beginning of the experiment the adrenergic vasoconstrictor response in the paw was elicited by sympathetic stimulation to insure function of the sympathetic innervation. Bretylium (15-20 mg/kg) was given acutely or guanethidine was administered on three consecutive days before the experiment to produce adrenergic blockade. Sympathetic nerve stimulation was carried out at 10 Hz for periods of 30 s until a maximal vasodilator response was attained. Atropine sulphate (0.25 mg/kg, i.v.) was administered and cholinergic blockade was determined by insuring that throughout the experiment the vasodilator response to 2 μg of acetylcholine injected intra-arterially was blocked. If acetylcholine produced significant vasodilatation, an additional dose of atropine (0.25 mg/kg) was given. Control vasodilator responses to three doses of histamine, two doses of bradykinin, and three frequencies of nerve stimulation were then elicited. In the experiments in which the H_2-receptor blocker, metiamide was administered, 4-methylhistamine, a selective H_2-agonist (Black *et al.*, 1972) was also given. Four groups of experiments were carried out. In one group ($n = 5$) bretylium was administered and the control vasodilator responses elicited. Burimamide was injected (5 mg/kg, i.v.) and infused (4 mg, i.a.) over a 10-15 min period and the vasodilator responses repeated once or twice after its administration. Tripelennamine was then administered (2.5-5 mg/kg, i.v.) and infused (4 mg/kg, i.a.) and the responses again repeated. A second group of experiments ($n = 6$) were similarly carried out in guanethidine-treated dogs. In a third group of experiments ($n = 5$), tripelennamine (2.5 mg/kg, i.v. and 4 mg, i.a.) was administered before burimamide, and then burimamide (8 mg, i.a.) was given. These experiments were carried out in guanethidine-treated dogs. Vasodilator responses were obtained after tripelennamine alone and then after burimamide. In this and the last group of experiments the sequence of eliciting the responses to histamine and nerve stimulation were alternated from one experiment to the other. In another group of guanethidine-treated dogs ($n = 6$) metiamide was infused intravenously at 0.25-5 mg kg^{-1} min^{-1}, after administration of tripelennamine.

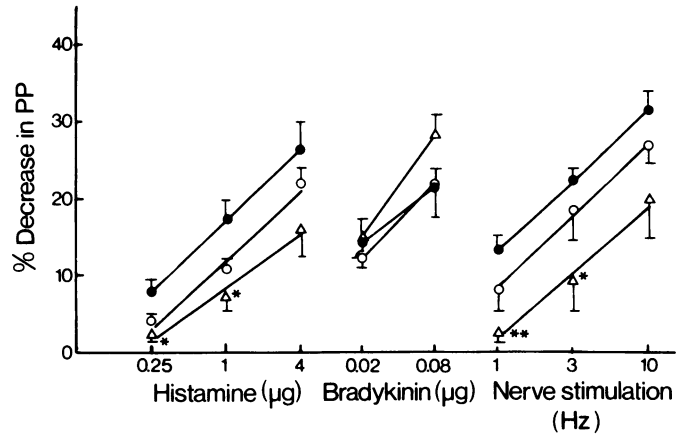

Figure 1 Dose-response curves to histamine and bradykinin, and frequency-response curves to nerve stimulation in (●) control period; (○) after burimamide (5 mg/kg, i.v. and 4 mg, i.a.) and (△) after tripelennamine (2.5-5 mg/kg, i.v. and 4 mg, i.a.). Values are mean of 5 experiments. Vertical bars show s.e. mean. $*P < 0.05$, $**P < 0.01$. PP = paw perfusion pressure.

Adrenergic vasoconstriction

Vasoconstrictor responses to three frequencies of sympathetic nerve stimulation and to three doses of noradrenaline injected intra-arterially were elicited in the control period and during an early and late period after burimamide alone, and after tripelennamine. In these experiments ($n = 4$) 5 mg/kg of burimamide and 2.5 mg/kg of tripelennamine were administered intravenously and 4 mg of each were given in addition intra-arterially.

Results are given as the mean with s.e. mean. For statistical analysis, the paired t test was used and differences were considered significant if $P < 0.05$. In order to minimize the influence of changes in perfusion pressure induced by the antihistamines on vasodilator responses, each response is expressed as a percentage of the control perfusion pressure just before the response.

Drugs

Histamine diphosphate (K and K Laboratories, Inc.); bradykinin (Nutritional Biochemicals); acetylcholine bromide (Eastman Organic Chemicals); atropine sulphate (K and K Laboratories, Inc.); noradrenaline bitartrate, as base (Winthrop Laboratories); 4-methylhistamine, burimamide and metiamide (Smith, Kline & French); bretylium tosylate (Burroughs Wellcome Co.); and tripelennamine and guanethidine, used as hydrochloride and sulphate salts (Ciba-Geigy Corp.). Burimamide and metiamide were solubilized with 1 or 2 M HCl and employed as the hydrochloride salts as recommended by Smith, Kline & French Labs.

Results

Effect of burimamide and tripelennamine on sustained vasodilatation

In this series of experiments bretylium (15-20 mg/kg) and atropine (0.25 mg/kg) were administered to block adrenergic vasoconstrictor and cholinergic vasodilator responses to sympathetic nerve stimulation, respectively. Vasodilator responses elicited in the paw by histamine, bradykinin and sympathetic nerve stimulation in the control period, after burimamide (5 mg/kg, i.v. and 4 mg, i.a.) and after both burimamide and tripelennamine (2.5-5 mg/kg, i.v. and 4 mg, i.a.) are shown in Figure 1. After the H_2-blocker (during the interval of 60-90 min following its administration), there were small decreases in the vasodilator responses to histamine and nerve stimulation, but no change in the response to the internal control, bradykinin. At earlier intervals some non-specific depression by burimamide was noted as evidenced by a reduction in the response to bradykinin. After the subsequent administration of tripelennamine, there were further decreases in the responses to histamine and nerve stimulation which were now statistically significant. No reduction was seen in the response to bradykinin. Tracings from a representative experiment demonstrating these effects are shown

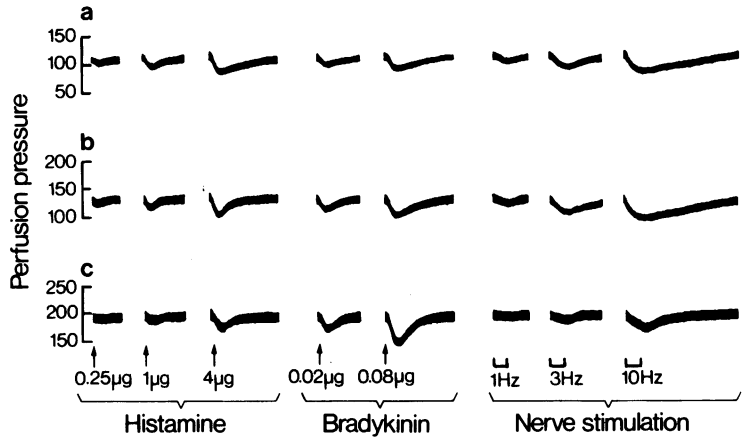

Figure 2 Responses of paw perfusion pressure (mmHg) to histamine, bradykinin and sympathetic nerve stimulation in (a) control period, (b) after burimamide (4 mg, i.a. and 5 mg/kg, i.v.) and (c) after tripelennamine (4 mg, i.a. and 5 mg/kg, i.v.). The responses were obtained during the period of 61 to 75 min after administration of burimamide and 18 to 30 min after tripelennamine. The period of sympathetic stimulation denoted by vertical lines is 30 seconds.

in Figure 2. Tripelennamine typically caused an increase in perfusion pressure and this vasoconstrictor effect tended to augment the absolute vasodilator responses. However, when the responses are expressed as a percentage of the control perfusion pressure, it is apparent that only the response to bradykinin remained the same, whereas responses to histamine and nerve stimulation were decreased.

Effect of burimamide or metiamide and tripelennamine on sustained vasodilatation in guanethidine-treated dog

In the experiments carried out in guanethidine-treated dogs, three doses of guanethidine, 5, 10 and 15 mg/kg (as the salt) were given intramuscularly in four and 2.5, 5 and 5-10 mg/kg doses (as base) were given intramuscularly or subcutaneously in 12 dogs. As adrenergic effects were abolished because of guanethidine treatment, a vasodilator response was obtained immediately upon sympathetic nerve stimulation in these animals, and there was less tendency for the response to increase with repetitive intervals of stimulation, as occurred after bretylium. Atropine (0.25 mg/kg) was given in these experiments to block the cholinergic component of the response. Control vasodilator responses, responses obtained after burimamide, and those obtained after burimamide and tripelennamine are shown in Figure 3. Burimamide in this dose caused some reduction in responses to histamine, bradykinin and nerve stimulation, but the changes were not significant. The subsequent administration of tripelennamine blocked the responses to histamine to a similar degree as in the experiments with bretylium (Figure 1), whereas only the response to nerve stimulation at 1 Hz was reduced significantly in contrast to the results obtained in bretylium-treated dogs.

In the second group of experiments carried out in guanethidine-treated animals the sequence of administration of antihistamines was reversed. This was done since the possibility existed that the effect of burmamide may have worn off before tripelennamine was given. The results presented in Fig. 4 demonstrate the effect of tripelennamine (2.5 mg/kg, i.v. and 4 mg, i.a.) given prior to the infusion of burimamide (8 mg, i.a.). Tripelennamine caused a small reduction in the response to histamine, but no decrease in the effect of nerve stimulation or bradykinin. The blockade of the response to histamine was of a lesser degree than that seen when burimamide had been given before tripelennamine (Figure 1), which indicates that the effect of the H_2-receptor blocker persisted in the first group of experiments. Burimamide administered after the H_1-receptor blocker caused a significant reduction in the responses to the lower doses of histamine during the period of 4.5-36 min after its administration. There was still evidence of histamine blockade at a later period (46-72 min) not shown in the figure. The responses to nerve stimulation and bradykinin were somewhat increased in this group of experiments after burimamide, but except for the response to

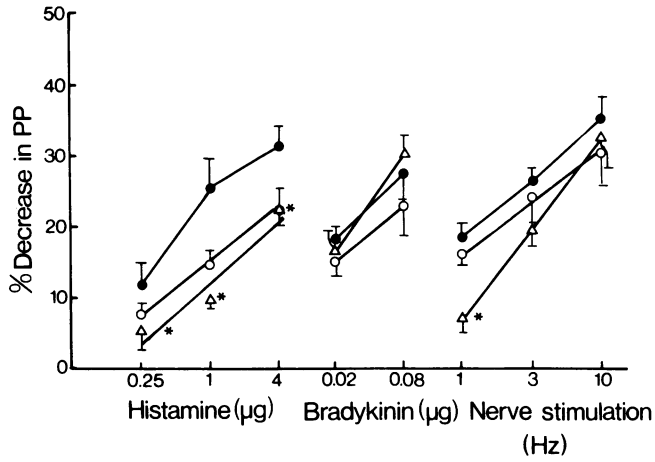

Figure 3 Dose-response and frequency-response curves in guanethidine-treated animals as in Figure 1. Values are mean of 6 experiments. Vertical bars show s.e. mean.

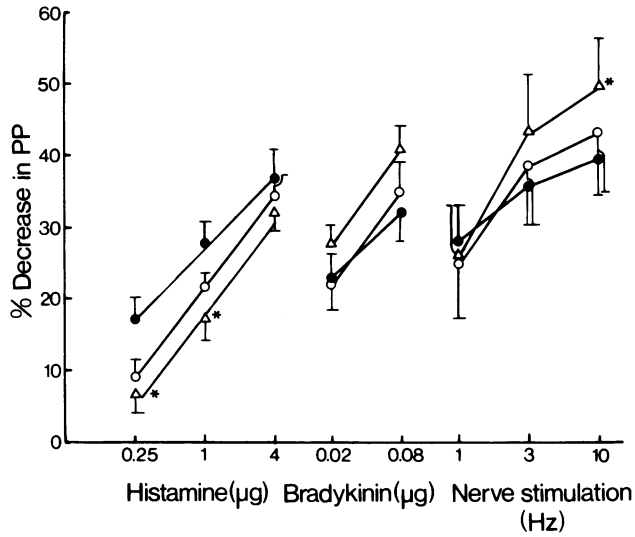

Figure 4 Dose-response and frequency-response curves in guanethidine-treated animals in (•) control period; (○) after tripelennamine (2.5 mg/kg, i.v. and 4 mg, i.a.) and (△) after burimamide (8 mg, i.a.). Values are mean of 5 experiments. Vertical bars show s.e. mean.

stimulation at 10 Hz were not significantly changed from the control.

The experiments in which metiamide (0.25-0.5 mg/kg) was infused continuously after the administration of tripelennamine were the most revealing with respect to the relative blockade of the responses to histamine and sustained vasodilatation. Responses to all three doses of histamine were markedly reduced and the responses to 4-methylhistamine were nearly abolished during infusion of metiamide (Figure 5). In contrast to this potent blocking effect on responses to histamine and methylhistamine, metiamide exerted no blocking action on the vasodilator responses to the internal control, bradykinin, or nerve stimulation. The latter responses tended to be somewhat larger in magnitude after tripelennamine and metiamide because of the vasoconstriction brought about by the antihistamines. Tripelennamine alone as in the

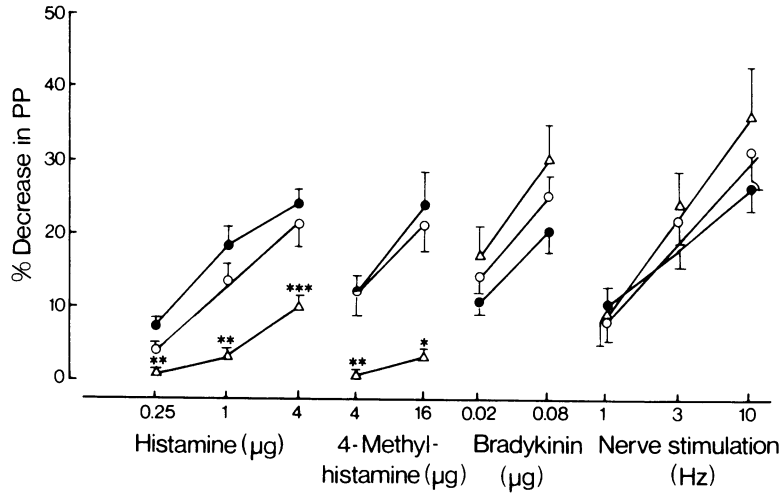

Figure 5 Dose-response curves to vasodilator agonists, including 4-methyl-histamine; and frequency-response curves obtained in guanethidine-treated animals in (●) control period; (○) after tripelennamine (2.5 mg/kg, i.v. and 4 mg, i.a.) and (△) during metiamide infusion (0.25-0.5 mg/kg, i.v.). Values are mean of 5 or 6 experiments. Vertical bars show s.e. mean. $*P < 0.025$, $**P < 0.01$, $***P < 0.001$.

above described series of experiments had only a slight blocking effect on the response to histamine, and interestingly had no effect on the response to methylhistamine. The sustained vasodilator responses were similarly unaffected by tripelennamine when it was administered before an H_2-blocker in these guanethidine-treated dogs.

Table 1 shows the blood pressure and perfusion pressure values recorded in the control period, after burimamide, after metiamide and after tripelennamine for the four groups of experiments. Blood pressure remained stable and above 100 mmHg throughout these experiments. After an initial vasodilator response caused by the infusion of burimamide and to a lesser degree tripelennamine (not in Table), the perfusion pressure increased after administration of the antihistamines. Vasoconstriction was more pronounced after tripelennamine than following administration of burimamide or metiamide. Since release of catecholamines from adrenergic nerve endings does not occur after guanethidine, the vasoconstrictor action of tripelennamine is due either to a direct effect on vascular smooth muscle or to sensitization to the effect of circulating catecholamines released from the adrenal medulla.

Table 1 Blood pressure (BP) and perfusion pressure (PP) in control period, and after antihistamines in bretylium- and guanethidine (Guan) -treated animals

Blocker	*Period 1*	*BP*	*PP*	*Period 2*	*BP*	*PP*	*Period 2*	*BP*	*PP*
Bretylium ($n = 5$)	Control	140 ± 7	124 ± 14	Burim	138 ± 12	118 ± 8	Trip	137 ± 10	172 ± 9
Guan ($n = 6$)	Control	124 ± 4	109 ± 16	Burim	113 ± 6	122 ± 21	Trip	116 ± 9	164 ± 20
Guan ($n = 5$)	Control	114 ± 2	85 ± 16	Trip	127 ± 5	114 ± 14	Burim	117 ± 4	134 ± 11
Guan ($n = 5$ or 6)	Control $n = 6$	116 ± 9	94 ± 14	Trip $n = 5$	126 ± 12	143 ± 17	Met $n = 5$	135 ± 8	170 ± 14

Burim = burimamide, Trip = tripelennamine, Met = metiamide.

Table 2 Responses to noradrenaline (NA) and nerve stimulation (NS) in control period, after burimamide and after tripelennamine (n = 4)

		Control	*Burimamide (5 mg/kg, i.v. 4 mg, i.a.)*		*Tripelennamine (2.5 mg/kg, i.v. 4 mg, i.a.)*	
			Early	*Late*	*Early*	*Late*
NA	0.2 μg	62 ± 5	12 ± 3**	42 ± 7	65 ± 15	92 ± 16
	0.4	94 ± 8	21 ± 4***	76 ± 16	102 ± 18	162 ± 49
	0.8	144 ± 25	41 ± 10*	133 ± 44	186 ± 56	292 ± 125
NS	1 Hz	121 ± 20	48 ± 4*	100 ± 20	99 ± 20	125 ± 23
	3	160 ± 27	113 ± 16	174 ± 23	171 ± 28	214 ± 17*
	10	228 ± 31	196 ± 23	242 ± 21	303 ± 24	366 ± 55**

Values are mmHg, mean with s.e. mean. *$P < 0.05$, compared to control, **$P < 0.01$, compared to control, ***$P < 0.001$.

Effect of burimamide and tripelennamine on adrenergic vasoconstrictor responses

Vasoconstrictor responses to three doses of noradrenaline administered intra-arterially and three frequencies of nerve stimulation in the control period, after burimamide (5 mg/kg, i.v. and 4 mg, i.a.) and after the subsequent administration of tripelennamine (2.5 mg/kg, i.v. and 4 mg, i.a.) are given in Table 2. There was a marked decrease in the responses to noradrenaline and nerve stimulation early (2-16 min) after burimamide. The responses eventually returned to the control level during a later period following burimamide (87-101 minutes). Tripelennamine brought about potentiation of the responses to noradrenaline and nerve stimulation in the early period (7-35 minutes). This effect was even greater and statistically significant for the responses to 3 and 10 Hz during the later period (60-85 min) after tripelennamine.

Discussion

Administration of either the H_1-receptor blocking agent, tripelennamine or the H_2-receptor blocking agent, burimamide alone caused only a small decrease in the vasodilator response to histamine in the cutaneous vasculature of the dog's paw. Burimamide exhibited a non-specific depressant effect immediately after its administration, which was most apparent when it was given in a high dose before tripelennamine. This depressant effect was avoided by employing only the intra-arterial dose of 8 mg of burimamide or alternatively by the continuous intravenous infusion of metiamide. The presence of H_2-receptors in the paw vasculature was clearly demonstrated in these experiments by the marked reduction in the response to histamine and almost complete abolition of the response to 4-methylhistamine during infusion of metiamide following tripelennamine. Burimamide also caused significant blockade of the response to histamine after tripelennamine; however, the antagonism was not as complete as that produced by metiamide after tripelennamine. Stimulation of H_2-receptors by histamine does not completely account for the vasodilator response to this agonist in the paw. When tripelennamine was administered subsequent to burimamide, it too produced a very significant histamine blockade which suggests that H_1-receptors are also present. The weak blocking effect on the response to histamine in the paw of either burimamide or tripelennamine when given alone is similar to the finding made in the cat by Black *et al.* (1972) when examining the blood pressure response to histamine. In that study when mepyramine, an H_1-blocker, was administered before burimamide, the subsequent administration of the H_2-blocker was effective in antagonizing the depressor response to histamine. When given alone, burimamide had a negligible blocking effect.

Large doses of guanethidine were employed in the present investigation to produce a complete adrenergic blockade. The sustained vasodilator response obtained in the guanethidine-treated dog was not consistently reduced by either antihistamine or by their combined administration. The response to stimulation at 1 Hz was depressed by tripelennamine in one group of guanethidine-treated animals, but this may represent a nonspecific effect. The results obtained in the animals given bretylium differed from those obtained when guanethidine was employed. A substantial reduction in the sustained vasodilator response was found after combined administration of the H_1- and H_2-receptor blockers in the animals in which bretylium was used to produce adrenergic

blockade. We attribute the apparent blockade of the sustained vasodilator response in the bretylium-treated animal to potentiation of a residual adrenergic vasoconstrictor effect by tripelennamine. Bretylium, unlike guanethidine, failed to eliminate the adrenergic response completely, and this was masked by the overriding vasodilation which occurs after bretylium. As shown in this study, tripelennamine is capable of potentiating the vasoconstrictor response to noradrenaline and adrenergic nerve stimulation, presumably by its blocking action on adrenergic uptake (Isaac & Goth, 1967). This effect may also have accounted for the reduction of the sustained vasodilator response by antihistamines that was observed in earlier investigations (Zimmerman, 1966; Rolewicz & Zimmerman, 1972). From the results of the present study it seems unlikely that histamine is the mediator of sustained vasodilatation in the dog's paw.

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