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Research Papers

Pharmacological properties of some imidazole derivatives occurring in nature

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Some naturally occurring histamine derivatives such as monomethylhistamine [4-(2-methylaminoethyl)imidazole], dimethylhistamine [4-(2-dimethylaminoethyl)-imidazole], spinaceamine (4,5,6,7-tetrahydroimidazo[5,4-c]pyridine) and 6-methyl-spinaceamine, and the quaternary ammonium base of histamine [4-(2-trimethyl-aminoethyl)imidazole] hitherto unknown in nature, were submitted to a pharmacological examination. The actions of monomethylhistamine resembled closely those of histamine. Dimethylhistamine was 2 to 20 times less active than histamine and showed some weak "nicotinic" effects. Trimethylhistamine had about 1% of the activity of histamine but showed a potent "nicotinic" activity on the preparations examined. Spinaceamine and 6-methylspinaceamine were virtually inactive. The importance of the *N'*-methylhistamines which behave similarly to the methyl derivatives of 5-hydroxytryptamine is discussed.

IN a systematic study of biologically active amines in the amphibian skin, skin extracts of some South-American *Leptodactylinae* were found to contain large amounts of imidazole derivatives. Using paper chromatography and biological assay of the natural compounds compared with the corresponding synthetic compounds, it was possible to identify not only histamine, monomethylhistamine and dimethylhistamine, but also two hitherto undescribed imidazole-*c*-pyridine derivatives: spinaceamine and 6-methylspinaceamine (Erspamer, Vitali, Roseghini & Cei, 1963).

Much has been written about the pharmacological properties of the *N'*-methylhistamine derivatives but many discrepancies exist in the reported data. Dale & Dudley (1921) found the monomethylhistamine to have 1/200th the activity of histamine on the cat blood pressure and 1/80th its activity on the guinea-pig uterus. In contrast to the results of Fränkel & Zeimer (1920), they also observed that the "imidazolisopiperidine" (spinaceamine) had only 1/1500th of the activity of histamine on uterine muscle of guinea-pig and practically no action on the blood pressure of cats. Fargher & Pyman (1921) claimed that monomethylhistamine has a negligible histamine-like action (about 1/100) and later, Garforth & Pyman (1935) found it to be approximately as active as histamine on the guinea-pig uterus. Vartiainen (1935) found the monomethyl derivative twice as potent as histamine on the guinea-pig uterus and intestine. Huebner, Turner & Sholz (1949) observed that both mono- and di-methylhistamine exhibited 75% of the oxytocic activity of histamine. Burger (1960) claimed the monomethyl derivatives to be only one half as active as histamine in the cat, but to exert twice its action on the blood pressure of the guinea-pig. Ingle & Taylor (1963) found dimethylhistamine and other imidazolealkylamines to be 10 to 100 times

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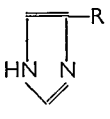
less potent than histamine. In addition, Tabor (1954) says that monomethylhistamine "has not been reported as a naturally occurring substance", but Kapeller Adler & Iggo (1957) have found both mono- and dimethyl derivatives in human urine. While the ring *N*-methylation was considered one of the main routes of histamine metabolism by many (Schayer, 1956, 1959; Schayer & Karyala, 1956; Brown, Tomchick & Axelrod, 1959), Gaddum recently (1962) pointed out the possibility that the most important mechanism by which histamine is inactivated in the body was the methylation of the side chain amino-group. To complicate matters, terminology is sometimes incorrect or incomplete since references are made to "methyl derivatives" but which kind is not stated.

Because of the discrepancies and also of the part that *N'*-methylhistamines are apparently destined to assume in the metabolism of histamine, especially after the discovery of new natural imidazole derivatives, it seemed opportune to submit the whole series of *N*-'methylated histamine derivatives (including the quaternary ammonium base of histamine hitherto unknown in nature) to a thorough pharmacological study. This we report.

Compounds. Histamine, I, R = $CH_2 \cdot CH_2 \cdot NH_2$ (2HCl); *N'*-methylhistamine[4-(2-methylaminoethyl)imidazole], I, R = $CH_2 \cdot CH_2 \cdot NH(Me)$ (HCl); *N'N'*-dimethylhistamine [4-(2-dimethylaminoethyl)imidazole], I, R = $CH_2 \cdot CH_2 \cdot NMe_2$ (HCl); *N'N'N'*-trimethylhistamine [4-(2-trimethylaminoethyl)imidazole, I, R = $CH_2 \cdot CH_2 \cdot NMe_3Cl$ (HCl): spinaceamine 4,5,6,7-tetrahydromidazo[5,4-*c*]pyridine; II, R = H; 6-methylspinaceamine 6-methyl-4,5,6,7-tetrahydroimidazo[5,4-*c*]pyridine; II, R = Me.



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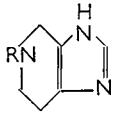

II

Dr. Vitali prepared the mono-, di- and trimethyl derivatives and both spinaceamines. Samples of histamine, nicotine and hexamethonium, were purchased from Merck and Recordati respectively. Leptodactyline and murexine were natural compounds prepared in our Institute. Weights of the compounds are quoted in terms of their free bases.

Pharmacological methods

HISTAMINE-LIKE EFFECTS

Guinea-pig ileum. Drugs were tested in a normal Krebs solution on the guinea-pig ileum prepared in the usual manner, and after treatment with atropine (10^{-7}), mepyramine (2 to 10,000 ng/ml) and hexamethonium (10 to 100 μg/ml) respectively.

Capillary permeability. This was tested on human and guinea-pig skin vessels.

Human skin vessels. Drugs were injected on the flexor surface of the forearm of 12 human volunteers as described to us by De Caro (1963). The dose of the test substance varied between 25 ng and 25 μg, the volume was always 0·1 ml. The size of the pomphus and the intensity of the erythema were examined.

Guinea-pig skin vessels. The methods was based on that of Miles & Miles (1952). After clipping away the hair, the animals were injected intravenously with 1·2 ml/kg of a 5% solution of Pontamine Sky Blue 6 BX in saline. 1 hr later the test drugs were injected intradermally in a volume of 0·1 ml. Doses of histamine varied between 50 and 500 μg. The effects of the compounds were evaluated for their ability to produce an accumulation of dye at the site of injection.

Guinea-pig bronchoconstriction. The technique used by Collier, Holgate, Schachter & Shorley (1960) was followed.

Blood pressure. Animals were anaesthetised using chloralose (90–100 mg/kg), pentobarbitone (30 mg/kg) or urethane (1–1·3 g/kg) each given intravenously. Cats and dogs received intravenous injections into the femoral vein, rabbits into the marginal vein of the ear and rats into the jugular vein after a light ether anaesthesia. Blood pressure was measured from the femoral or carotid artery by a mercury manometer.

UNSPECIFIC EFFECTS

Frog rectus abdominis and leech dorsal muscle were prepared in the usual manner. Rat diaphragm was prepared as described by Bülbring (1946). Cat sciatic nerve-gastrocnemius preparation, cat nictitating membrane and spinal cat, were according to Burn (1950).

Results

HISTAMINE-LIKE ACTIONS

The activity of derivatives as compared with histamine on various preparations is shown in Table 1.

Guinea-pig ileum. The response of the guinea-pig ileum to all the compounds was not modified after atropine (10^{-7}). After mepyramine (2 ng/ml), histamine and its monomethyl derivatives were antagonised to

TABLE 1. HISTAMINE-LIKE EFFECTS OF THE IMIDAZOLE DERIVATIVES COMPARED WITH HISTAMINE

The activity of histamine is arbitrarily taken as 100. The activities of the other drugs are expressed as percentages. ϕ = the compound is inactive or at least over 1000 times less active than histamine. Sign — = not tested. Values represent the mean of the values obtained from 3 to 6 different preparations of each test.

	H	MMH	DMH	TMH	Sp	MSp
Guinea-pig ileum	100	85	45	1	0·03	0·05
Human skin vessels	100	95	20	0·40	0·10	0·08
Guinea-pig bronchoconst.	100	90	20	—	—	—
Guinea-pig skin vessels	100	65	5	<1	ϕ	ϕ
Blood pressure						
Dog	100	60–65	45–55		ϕ	ϕ
Cat	100	75	35		0·55	0·15
Rat	100	75	35	30	—	—
Rabbit	100	85	35	(75)	<0·2	<0·2

H = Histamine; MMH the mono-, DMH the di-, and TMH the trimethyl derivatives. Sp = spinaceamine; MSp = methylspinaceamine.

the same extent. This is in accordance with the observations of Schild (1947). The dimethyl and especially the trimethyl derivative were affected to a lesser degree than histamine by the antihistamine agent. The negligible activity shown by spinaceamine is in accordance with observations by Dale & Dudley (1921) on the guinea-pig uterus and cat blood pressure.

Human skin vessels. Our results with monomethylhistamine agreed with Vartiainen's data (1935), although they were obtained with a slightly different technique. The dimethyl derivative we found to be much less active than did Vartiainen. The trimethyl derivative showed a weak but typical histamine-like effect.

Guinea-pig skin vessels. In this test the activity of monomethylhistamine came close to that exhibited on other preparations while dimethylhistamine showed little activity.

Guinea-pig bronchoconstriction. The ratio of activity between histamine and its methyl derivatives was similar to that obtained in above preparations.

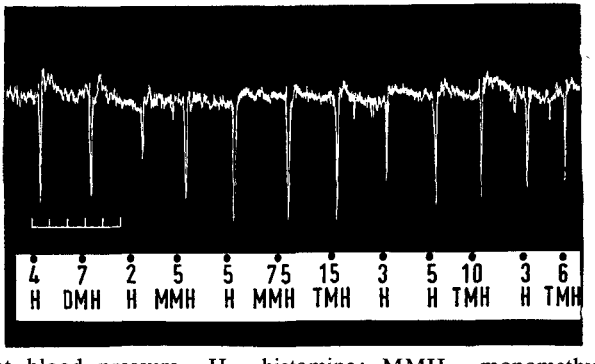

FIG. 1. Rat blood pressure. H = histamine; MMH = monomethylhistamine; DMH = dimethylhistamine; TMH = trimethylhistamine. Doses in μg. Time in min.

Blood pressure. Dog. On dog blood pressure the action of the mono- and di-methyl derivatives resembled that of histamine (60–65 and 45–55% as active respectively). Trimethylhistamine did not exhibit any histamine-like action in doses up to 25–50 μg/kg. At 100–200 μg/kg, it caused an hypertensive response similar to that produced by leptodactyline but 5 to 10 times less intense.

Cat. The cat behaves essentially like the dog in its blood pressure responses to the derivatives. The two spinaceamines were not completely inactive on this test.

Rabbit. The effects of histamine and of the methylhistamines were erratic. Sometimes they caused hypotension, sometimes hypertension and sometimes a diphasic response. The behaviour of mono- and di-methylhistamine was similar to that of histamine. Trimethylhistamine usually produced an initial fall of blood pressure followed by a more sustained hypertension. This diphasic response was not modified by mepyramine or by atropine.

Rat. In rats anaesthetised with pentobarbitone, the ratio between histamine and its methyl derivatives was the same at any dose level. In this species, trimethylhistamine mimicks histamine in its effect on blood pressure. The transient hypotension it produced was similar to that produced by histamine and seldom was it followed by a small short-lived rise of blood pressure (Fig. 1).

OTHER ACTIONS

Trimethylhistamine displayed, as expected, "nicotinic" actions on a number of biological preparations.

The "nicotinic" effects of dimethylhistamine were much less evident. Table 2 shows the activity of trimethylhistamine in comparison with that of leptodactyline, murexine, nicotine and adrenaline.

TABLE 2. NICOTINE-LIKE EFFECTS OF DIFFERENT DRUGS COMPARED WITH TRIMETHYLHISTAMINE

The figures indicate the number of moles of the different compounds required to give the same effect as 1 mole of trimethylhistamine. Values represent the mean of the values obtained from 2 to 6 different preparations of each test.

	TMH	Nic.	Lep.	Mur.	Ad.
Guinea-pig ileum	1	1			
Frog rectus	1	(0·75)	0·003	0·12	
Leech dorsal muscle	1	0·25			
Rat diaphragm	1	0·45			
Cat gastrocnemius	1		0·06	(0·20)	
Cat nictitating membrane	1	1·5	0·20		0·04
Cat, spinal	1	0·75	0·15		0·025
Dog blood pressure	1		0·16		

Nic. = nicotine; Lep. = leptodactyline; Mur. = murexine; Ad. = adrenaline.

Guinea-pig ileum. The activity ratio trimethylhistamine : nicotine which in the normal Krebs solution was 1 : 1, became 4 : 1 after mepyramine 2 ng/ml and 7 : 1 after mepyramine 100 ng/ml hence with this dose of the antihistamine, the trimethyl derivative was only 14% as active as nicotine. On the other hand, after hexamethonium, 10 μg/ml, the activity ratio trimethylhistamine : nicotine became 1 : 5 and it rose to 1 : 8 after

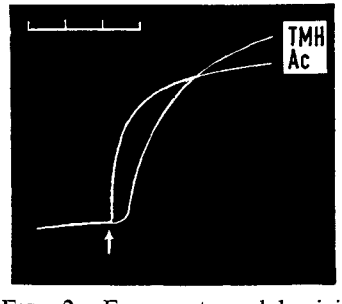

FIG. 2. Frog rectus abdominis preparation. At arrow 0·3 μg/ml acetylcholine (Ac) and after washing and relaxation of the muscle, 15 μg/ml TMH. Time in min.

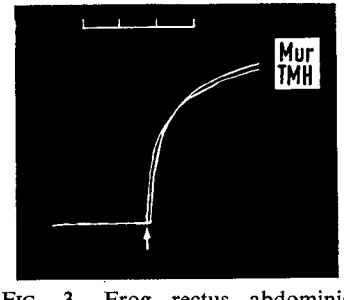

FIG. 3. Frog rectus abdominis preparation. At arrow 1·5 μg/ml murexine (Mur) and after washing and relaxation of the muscle, 10 μg/ml TMH. Time in min.

hexamethonium 100 μg/ml. With this dose, the activity ratio trimethylhistamine: histamine, which in the normal Krebs solution was 100: 1, became 170: 1.

Frog rectus abdominis. Trimethylhistamine caused, in this muscle, a contracture which closely resembled that produced by leptodactyline and murexine (Fig. 3) but was different from that produced by acetylcholine (Fig. 2) and nicotine. To give an approximate idea of the relative activities of these drugs, the heights of the contractions were compared after a fixed time. The minimum active dose of trimethylhistamine was 1 to 3 μg/ml and there was a satisfactory dose/response relationship. Tubocurarine showed a strong antagonistic action: after 0·5 μg/ml the contracture provoked by 25 μg/ml of the histamine derivative was reduced by 70 to 90% of the control; after tubocurarine, 1 μg/ml, the effect of the derivative was completely abolished. The dimethyl derivative showed approximately 2% of the activity of trimethylhistamine. As shown in Fig. 4 the contractures provoked by the two histamine derivatives were qualitatively identical.

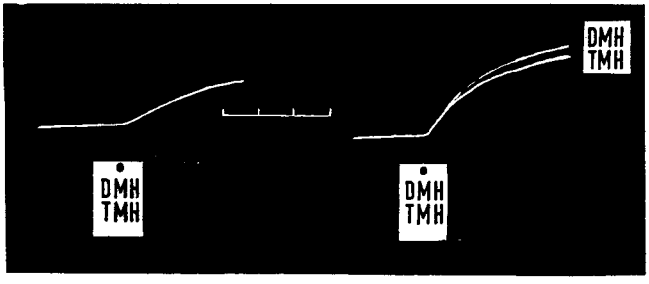

FIG. 4. Frog rectus abdominis preparation. At first dot 0·2 mg/ml DMH and 4 μg/ml TMH. At second dot 0·4 mg/ml DMH and 8·5 μg/ml TMH. Time in min.

Leech dorsal muscle. Trimethylhistamine contracture was similar to that elicited by nicotine. Eserine salicylate, 0·2 μg/ml, potentiated the response of both the derivative and nicotine by 30 to 50% of the control.

Rat diaphragm. This preparation showed little sensitivity to trimethylhistamine. The threshold blocking dose was 20 to 30μg/ml. Whereas the

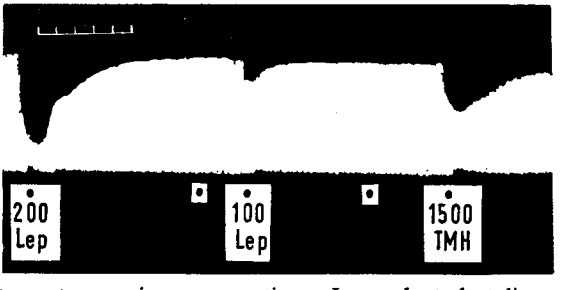

FIG. 5. Cat gastrocnemius preparation. Lep = leptodactyline; TMH = trimethylhistamine doses in μg/kg. Recording stopped for 15 min at unlabelled dots. Time in min.

derivative was about 50 times less potent than suxamethonium as a neuromuscular blocking agent, it was only 10 to 20 times weaker as a tubocurarine (0·5 μg/ml) antagonist.

Cat gastrocnemius. The action of trimethylhistamine in reducing twitches resembled that of leptodactyline (Fig. 5) though it was approximately 15 times weaker and only half as potent in antagonising tubocurarine (50 μg/kg). The minimum blocking dose was 200 to 400 μg/kg. Although the derivative was only 5 times less potent than murexine, its action was considerably more shortlived. It was approximately 1/20th as potent as suxamethonium. As with many other blocking agents, repeated administration of trimethylhistamine at 15 min intervals resulted in drug cumulation (Fig. 6). Dimethylhistamine was completely ineffective up to 1 mg/kg: higher doses could not be tested owing to its action on blood pressure.

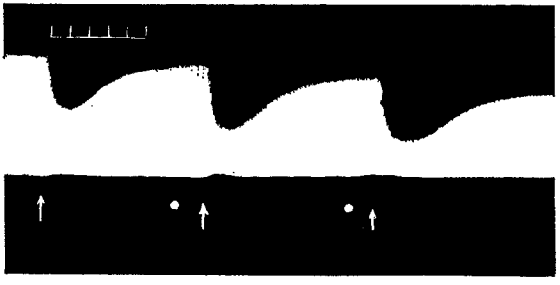

FIG. 6. Cat gastrocnemius preparation. At arrow 0·5 mg/kg TMH. Recording stopped for 15 min at dots. Time in min.

Cat nictitating membrane. The threshold dose of trimethylhistamine on this preparation was 50 to 75 μg/kg. The contraction elicited by 200 μg/kg was reduced by 50–70% after hexamethonium, 0·5 mg/kg, and completely abolished after 1 mg/kg.

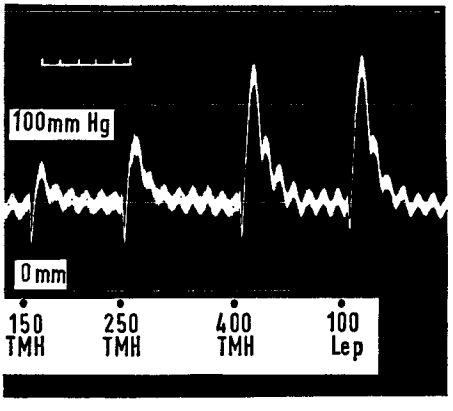

FIG. 7. Spinal cat preparation. Lep = leptodactyline; TMH = trimethylhistamine doses in μg/kg. Time in min.

Spinal cat. As with the normal cat, hypertension caused by trimethylhistamine was preceded by a small fall of the blood pressure which was not proportional to the dose administered. The rise of blood pressure was satisfactorily proportional to the dose (Fig. 7). Hypertension produced by 200 to 500 μg/kg, was reduced by about 30 to 40% after adrenalectomy. This may signify that only 30–40% of trimethylhistamine hypertension was due to release of medullary catecholamines.

Discussion

It appears from the experimental data that monomethylhistamine most closely resembled histamine in its action on blood pressure and on plain muscles, but its activity was always weaker than that of histamine.

Dimethylhistamine was 2 to 20 times less active than histamine. This tertiary amine showed a feeble nicotine-like activity on the frog rectus abdominis and on the guinea-pig ileum after mepyramine. At low doses, mepyramine inhibited the dimethyl derivative to a lesser degree than it did histamine.

Trimethylhistamine was characterised by the predominance of nicotine-like activity. Table 2 shows it to be the derivative quantitatively more closely related to nicotine, and murexine and leptodactyline to be more potent than either. As well as nicotine-like action, the trimethyl derivative had a histamine-like action, though this was less powerful than that of the two other methyl derivatives. This histaminic activity was demonstrated by the activity shown in the isolated guinea-pig ileum, in which the responses were modified after mepyramine and hexamethonium, and by the weak but specific histamine-like action exhibited on the human skin vessels.

The lack of activity of spinaceamine agrees with the findings by Dale & Dudley (1921). Its 6-methyl derivative behaved similarly.

We thus conclude that stepwise *N*-methylation in the side chain of the histamine molecule regularly reduces the histamine-like activity and this negative effect increases with the number of the methyl groups. Linkage of the side chain to the 5 position of the imidazole nucleus through a *N*-methyl group, as occurring in spinaceamine and in 6-methylspinaceamine, completely abolishes the activity. The reduction of histamine activity was accompanied by the appearance of a nicotinic activity, as with the di- and especially the trimethylhistamine.

Most of our results are similar to those obtained by others. Some discrepancies may be explained, at least partially, with the different methods of chemical preparations of the compounds. Sometimes, as with Frankel & Zeimer's (1920) findings for spinaceamine, and those of Fargher & Pyman (1921) for monomethylhistamine, the chemical synthesis probably gave contaminated products. Quantitative differences may also depend on the different animal species used for the pharmacological examination.

The relationship between chemical structure and pharmacological activity observed with histamine derivatives may bear comparison with observations

on 5-hydroxyindolealkylamines (Erspamer, 1952; Bertaccini & Zamboni, 1961) namely: progressive decay of 5-hydroxytryptamine activity with increasing introduction of *N*-methyl groups with the final appearance of nicotinic actions; loss of activity with linkage of the side-chain to the indole nucleus to form a tricyclic system—pyrrole [3,4,5-*d,e*]quinoline (Märki, Robertson & Witkop, 1961).

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