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INHIBITION OF THE METHYLATION OF HISTAMINE IN CAT BRAIN

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It has been known for some years that ring-*N*-methylation constitutes an important pathway for the catabolism of histamine in mammals (Schayer, 1959). Recent studies indicate that brain tissue in particular has a great capacity to methylate histamine. This has been shown for the cat both *in vitro* (Brown, Tomchick & Axelrod, 1959; White, 1959) and *in vivo* (White, 1960). Methylation seems to be the major pathway for histamine catabolism in the brain. There is little information on the inhibition of histamine methylation *in vivo*, although a number of substances have been found to inhibit a purified preparation of the histamine–methylating enzyme *in vitro* (Brown *et al.* 1959; Lindahl, 1960). To the author's knowledge no experiments have been carried out to study the inhibition of the methylation of histamine in the living brain.

The present experiments were performed to see if the formation of methylhistamine from histamine in the brain could be inhibited *in vivo* by two of the substances found to be effective *in vitro*, namely chlorpromazine and cupric ions (Brown *et al.* 1959; Lindahl, 1960). It will be shown that both inhibit the methylation of histamine in the cat's brain.

METHODS

Cats of either sex weighing 2·5–3·5 kg were anaesthetized with Na pentobarbitone intraperitoneally. Animals pre-treated with chlorpromazine received 30 mg/kg and the others 45 mg/kg. The trachea was cannulated and blood pressure recorded by a mercury manometer connected to a femoral artery.

Perfusion took place from the lateral ventricle to the aqueduct, using a Collison cannula for the cannulation of the lateral ventricle (Bhattacharya & Feldberg, 1958). The position of the aqueductal cannula was confirmed at the end of each experiment by dye injection and by dissection of the brain with the cannula in place. In all experiments the tip of the cannula was in the middle of the aqueduct, or in the caudal half of it. In no experiment was leakage of dye into the fourth ventricle observed. In the experiments where high concentration of cupric chloride (0·01 M) and of chlorpromazine (0·05 M) were present in the fluid infused, the effluent showed slight flocculation or opalescence, which disappeared immediately after the addition of a small amount of 0·1 N-HCl. In the other experiments the effluents were clear and colourless.

The perfusion fluid had the following composition (g/l.): NaCl 9·00, KCl 0·42 and $CaCl_2$ (anhydr.) 0·24. In the following pages this solution will be referred to as Ringer's solution.

The ventricles were first perfused for 30 min with Ringer's solution, or Ringer's solution containing one of the inhibitors, and then with the same solution containing 2 μg ^{14}C-histamine base/ml. during 40 min. The infusions were made with a motor-driven syringe at a constant rate of 0·11 ml./min and the total volume of the ^{14}C-histamine solution infused was 4·5 ml. At the end of the perfusion with ^{14}C-histamine the ventricles were perfused with 5 ml. of Ringer's solution at a rate of approx. 0·5 ml./min, to wash out radioactive substances. For analysis the combined effluents were used. The whole brain including the cerebellum and the medulla oblongata down to the foramen magnum was excised.

The methods for measuring ^{14}C-histamine and its radioactive metabolites by isotope dilution technique have been developed by Schayer and others and were similar to those used earlier (White, 1959, 1960). A modification in the treatment of the brain samples was used in the present experiments. The brain was homogenized, after preliminary mincing, in a glass homogenizer with approx. the double volume of 0·1 N-HCl. The homogenate was divided into portions (usually 8 to 10), carrier was added to each, and protein precipitated with trichloroacetic acid. The samples were then filtered and treated as described earlier (White, 1960). Only a brief summary of the procedures will be given here.

The following metabolites of histamine were studied: Methylhistamine (1-methyl-4-(β-aminoethyl)-imidazole), methylimidazoleacetic acid (1-methyl-imidazole-4-acetic acid), and imidazoleacetic acid (imidazole-4(5)-acetic acid).

Histamine was extracted into butanol, converted to pipsyl histamine and counted. Methylhistamine was extracted into chloroform and counted in the form of methylhistamine picrate. Imidazoleacetic acid and methylimidazoleacetic acid were separated from histamine and methylhistamine on a cation exchange column (Dowex-50). Pipsylimidazoleacetic acid and the picrate of methylimidazoleacetic acid were then prepared and counted. All determinations of imidazoleacetic acid were preceded by acid hydrolysis of the samples, and the figures for imidazoleacetic acid therefore denote the total amount, free and conjugated. All samples were recrystallized to constant radioactivity, three to six recrystallizations being needed in most cases. The samples of methylhistamine in the perfusate sometimes had to be recrystallized even more often, because of the huge excess of radioactive histamine.

The measurements of radioactivity were made under infinite-thickness conditions in a gas-flow counter with a background activity of 21–24 counts/min. At least 900 counts were taken of each sample after each recrystallization. Differences in counting rates smaller than those corresponding to 0·2–0·3% in the tables are not considered significant.

^{14}C-histamine dihydrochloride labelled in the 2-position of the imidazole ring was purchased from the Radiochemical Centre, Amersham, Bucks. After dilution with the standard amount of inert carrier histamine (66·4 mg of the dihydrochloride) and precipitation as the dipicrate, this ^{14}C-histamine gave 3200 counts/min/μg base, counted at infinite thickness in the flow counter.

Chlorpromazine was used in the form of the hydrochloride (AB Leo, Hälsingborg, Sweden). The figures for the doses of chlorpromazine refer to the salt. Copper was used in the form of $CuCl_2 . 2H_2O$, containing 37·3% copper.

RESULTS

The control experiments (Table 1) showed that collection of the effluent from the aqueduct instead of from the cisterna magna caused slightly less methylated histamine metabolites (methylhistamine and methylimidazoleacetic acid) to be formed than in the experiments reported earlier (White, 1960). The methylation that did occur was still great enough to provide a basis for experiments on the inhibition of histamine methylation.

It has recently been shown by Lindahl (1960) that cupric ions inhibit a purified preparation of a histamine-methylating enzyme *in vitro*. In seven experiments the effect of cupric chloride on the methylation of histamine in the cat's brain was studied (Table 2). The cupric salt was administered intravenously (Expts. 5 and 6) and by ventricular perfusion (Expts. 1–4), and also by both routes simultaneously (Expt. 7). In Expts. 5 and 6 of Table 2 cupric chloride, dissolved in 0·9 % NaCl solution, was infused into a femoral vein at a rate of 0·12 mg copper/min for 60 min before and also during the ventricular perfusion with ^{14}C-histamine. In these experiments the methylation of histamine (i.e. formation of methylhistamine and methylimidazoleacetic acid) was inhibited by approx. 35 %.

TABLE 1. Catabolism of ^{14}C-histamine in cat brain during perfusions from the lateral ventricle to the aqueduct. Percentage of infused ^{14}C-histamine recovered as histamine and metabolites

Expt. no.		Histamine	Methyl-histamine	Methyl-imidazole-acetic acid	Imidazole-acetic acid	Sum of methyl derivatives	Total recovery
1	Effluent	83	2·9	0·4	0·2	9·7	94
	Brain	0·4	3·5	2·9	0·2		
2	Effluent	89	2·2	0·3	0·1	8·5	98
	Brain	0·5	3·8	2·2	0·1		
3	Effluent	83	2·0	0·4	0·6	9·8	94
	Brain	0·3	3·5	3·9	0·2		
4	Effluent	83	2·2	0·1	0·3	9·2	94
	Brain	0·8	4·8	2·1	0·2		
5	Effluent	87	2·7	0·4	0·2	9·1	96
	Brain	0·2	3·5	2·5	0·2		

Greater inhibition was observed when the cupric chloride was added to the perfusion fluid (Expts. 1–4 of Table 2), 0·001 M inhibiting approx. 55 % and 0·01 M approx. 80 %. It can be seen that in the experiments showing the smallest formation of methylhistamine (Expts. 3 and 4 of Table 2) the brain contained more unchanged histamine than in the control experiments (Table 1).

In Expt. 7 of Table 2 cupric chloride was infused both intravenously (0·12 mg copper/min) and intraventricularly. This experiment showed an inhibition of the histamine methylation slightly greater than that observed after each treatment alone.

Comparison between the results with and without copper in the perfusion fluid would only be valid if the concentrations of ^{14}C-histamine were the same. The formation of a histamine-copper complex in the copper-containing perfusion fluid (Eichler & Meyer, 1948) might diminish the concentration of free histamine. In the experiments where cupric chloride in a concentration of 0·01 M was infused the fluid remaining in the syringe after the termination of the perfusions was assayed for histamine activity on the isolated guinea-pig ileum against a corresponding solution lacking

TABLE 2. The effect of cupric chloride on the catabolism of ^{14}C-histamine in cat brain during perfusions from the lateral ventricle to the aqueduct. Percentage of infused ^{14}C-histamine recovered as histamine and metabolites

Expt. no.	$CuCl_2$		Histamine	Methyl-histamine	Methyl-imidazole-acetic acid	Imidazole-acetic acid	Sum of methyl derivatives	Total recovery
1	10^{-3} M intraventricularly	Effluent	83	0·5	0·0	0·9	4·7	89
		Brain	1·0	3·2	1·0	0·0		
2	10^{-3} M intraventricularly	Effluent	83	0·5	0·1	0·2	3·5	87
		Brain	0·6	1·8	1·1	0·1		
3	10^{-2} M intraventricularly	Effluent	91	0·3	0·0	1·5	1·9	96
		Brain	1·8	1·5	0·1	0·0		
4	10^{-2} M intraventricularly	Effluent	88	0·5	0·0	0·2	1·8	92
		Brain	1·5	1·2	0·1	0·0		
5	Intravenously	Effluent	94	1·3	0·2	0·4	6·0	101
		Brain	0·6	2·9	1·6	0·2		
6	Intravenously	Effluent	87	1·5	0·2	0·2	5·7	93
		Brain	0·3	2·0	2·0	0·1		
7	10^{-3} M intraventricularly + intravenously	Effluent	93	0·2	0·0	0·3	3·4	97
		Brain	0·8	2·4	0·8	0·0		

TABLE 3. The effect of chlorpromazine on the catabolism of ^{14}C-histamine in cat brain during perfusions from the lateral ventricle to the aqueduct. Percentage of infused ^{14}C-histamine recovered as histamine and metabolites

Expt. no.	Chlorpromazine		Histamine	Methyl-histamine	Methyl imidazole-acetic acid	Imidazole-acetic acid	Sum of methyl derivatives	Total recovery
1	5×10^{-2} M intraventricularly	Effluent	74	0·3	0·0	0·2	7·1	84
		Brain	2·1	4·6	2·2	0·0		
2	5×10^{-3} M intraventricularly	Effluent	67	0·5	0·2	0·2	15	84
		Brain	2·0	10·4	3·5	0·0		
3	3×50 mg/kg intramuscularly	Effluent	82	1·8	0·2	0·1	5·7	89
		Brain	0·8	2·7	1·0	0·1		
4	3×50 mg/kg intramuscularly	Effluent	92	0·8	0·0	0·3	3·8	96
		Brain	0·3	1·5	1·5	0·0		
5	3×50 mg/kg intramuscularly	Effluent	84	1·2	0·1	0·3	6·6	91
		Brain	0·4	3·3	2·0	0·0		
6	$3 \times 12{\cdot}5$ mg/kg intramuscularly	Effluent	91	1·8	0·4	0·0	6·6	98
		Brain	0·2	2·0	2·4	0·2		
7	$3 \times 12{\cdot}5$ mg/kg intramuscularly	Effluent	86	1·6	0·2	0·3	6·7	93
		Brain	0·5	3·0	1·9	0·2		

copper, and also against a reference solution prepared from non-radioactive histamine. No difference could be detected between the radioactive solutions with and without copper. The concentration of histamine in the perfusion fluid differed less than 10% from the expected value (2 μg/ml.). It was concluded that the addition of copper did not influence the amount of free biologically active ^{14}C-histamine in the perfusion fluid.

When chlorpromazine was perfused through the ventricles (Expts. 1 and 2 of Table 3), some depression of the blood pressure occurred, and it was difficult to obtain a constant flow through the ventricular system. It would seem that ventricular perfusion was not a suitable method for the administration of high concentrations of chlorpromazine to the brain.

In five experiments (3–7 of Table 3) cats were given three intramuscular injections of chlorpromazine, receiving two injections on the day before the ventricular perfusion, and the third about 2 hr before the start of the ventricular perfusion. The rather large doses employed caused changes in the behaviour of the animals. They became drowsy and passive, and the gait was somewhat inco-ordinated, but they were conscious and their reactions seemed otherwise adequate. The values obtained for the catabolism of ^{14}C-histamine in the brain in these experiments indicated less formation of methylhistamine and methylimidazoleacetic acid than in the control experiments (Table 1).

DISCUSSION

The methylation of histamine can take place in several regions of the brain, as judged from *in vitro* experiments on minced tissue (White, 1959) and on purified enzyme preparations (Brown *et al.* 1959). Histamine perfused through the cerebral ventricles of anaesthetized cats has been shown to enter the brain tissue surrounding the ventricular cavities, and this uptake of histamine shows differences dependent on the structures of the ventricular wall, the highest concentrations of histamine being found in the grey matter lining the ventricles (Draškoci, Feldberg, Fleischhauer & Haranath, 1960). It may be assumed that other substances also present in the ventricular fluid enter these regions, and that in the present experiments cupric ions travel together with histamine in the brain tissue to reach the sites where the histamine is methylated.

It is evident from a comparison of Table 1 with previous findings (White, 1960) that results obtained by collection of the effluent from the aqueduct are more consistent than those obtained by collection from the cisterna magna. These two techniques differ in that the fourth ventricle and part of the subarachnoid space are perfused in the latter method, but not in the former. It is also evident that the aqueductal perfusions tend to give lower figures for the sum of methylated derivatives. This may be

explained by methylation in the cerebellum and the caudal part of the brain stem (Brown *et al.* 1959), which are not reached by the perfusion fluid when the aqueduct is cannulated. When the effluent is collected from the cisterna magna the cerebellum and the brain stem may be reached, in different experiments, by varying amounts of ^{14}C-histamine (Bhawe, 1958), and therefore variable amounts of ^{14}C-methylhistamine may be formed. Further, the drainage through the cisternal cannula of fluid containing the radioactive substances may vary. This could also explain the variable and low values for the total recovery in the cisternal perfusions.

The present experiments *in vivo* demonstrate that cupric chloride and chlorpromazine decrease the amount of methylhistamine and methylimidazoleacetic acid formed during the experiment. The experiments do not supply precise evidence as to the mechanism responsible for this effect. It should be noted that the doses employed were rather large, and that the drugs may influence various functions in the organism. The greatest inhibition of the methylation of histamine was observed when cupric chloride was perfused through the ventricles. In this case the possibility must be considered that the cupric salt so altered the qualities of the lining surfaces of the ventricles that less histamine penetrated into the brain tissue, thereby causing less methylated histamine derivatives to be formed. If this had been so, and the methylating capacity of the tissue was still intact, one would expect to find less unchanged histamine in the tissue. On the other hand, if the penetration of histamine into the tissue from the ventricles had been normal, but the methylating process inhibited, then one would expect to find more unchanged histamine in the tissue, and less methylated derivatives. It can be seen that in Expts. 3 and 4 of Table 2 the findings agree best with the latter alternative. Thus it seems likely that the limiting factor in the experiments with intraventricular copper is the methylating capacity of the brain tissue rather than the penetration of histamine into the tissue.

Cats pre-treated with intramuscular injections of chlorpromazine showed less methylation of histamine during aqueductal perfusions than the control animals. Chlorpromazine is present in the rat brain several hours after an intramuscular injection of the drug (Wechsler & Forrest, 1959). The amount found in the brain after a dose slightly larger than that employed in the present report corresponds to a concentration of chlorpromazine greater than that producing inhibition of histamine methylation *in vitro* (Brown *et al.* 1959). The complexity of action of chlorpromazine in the intact organism makes the interpretation of any observed effect of the drug difficult, but the available evidence would agree with the assumption that chlorpromazine inhibits histamine methylation in the brain by inhibition of the histamine-methylating enzyme.

SUMMARY

1. Cupric chloride inhibited the methylation of histamine in the brain after intravenous and intraventricular administration.

2. Chlorpromazine injected intramuscularly inhibited the methylation of histamine in the brain.

3. Possible mechanisms of these actions of copper and chlorpromazine are discussed.

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