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STRUCTURAL FEATURES OF POTENT INHIBITORS OF RAT KIDNEY HISTAMINE *N*-METHYLTRANSFERASE

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Abstract—Three antimalarial drugs amodiaquine, quinacrine and chloroquine were compared side-by-side with the antiseptic agent chlorhexidine and the neuromuscular blocker alcuronium for their capacity to competitively inhibit *in vitro* the activity of the enzyme histamine *N*-methyltransferase (HMT) from rat kidney over the concentration range 10^{-3}–10^{-8}. Amodiaquine was clearly the most potent HMT inhibitor followed by quinacrine, chlorhexidine, alcuronium and chloroquine. Investigation of the structure–activity relationships by examining space-filling models revealed marked similarities in the conformations of the arrangement of three N atoms in histamine and in each of the compounds tested.

Although the enzymatic–isotopic microassay for histamine as first described by Snyder *et al.* [1] was thought to be the most specific assay for this autacoid and to be the least susceptible to interference by other substances, an increasing number of compounds are being found which significantly inhibit or enhance the activity of histamine *N*-methyltransferase (HMT). Such compounds include close structural analogues of histamine such as N^{α}-methylhistamine and 2-methylhistamine [2], antimalarials, antihistamines, local anaesthetics and diuretics [3] and neuromuscular blockers (NMBs) [4]. The NMB alcuronium was almost as potent an inhibitor of HMT as SKF 91488, one of the most potent HMT inhibitors known [4, 5]. A general conclusion from these studies of competitive inhibitors of HMT was that compounds containing ammonium groups, and particularly diethylaminoethyl groups, were the most potent inhibitors [2, 4, 5–7].

While investigating the allergic release of histamine by chlorhexidine *in vitro* in a patient allergic to this compound, we found that the activity of rat HMT was significantly inhibited by the compound in the concentration range 10^{-7}–10^{-5} M. This finding led us to compare the activity of chlorhexidine with some of the most potent of the known inhibitors of HMT and to look closely at the structures of this compound, some antimalarials, alcuronium and SKF 91488 for a possible structural basis for the inhibitory activity.

MATERIALS AND METHODS

(a) *Materials*

Enzyme preparation. HMT was purified from male Sprague–Dawley rat kidney as previously described [4].

Reagents. Histamine dihydrochloride, chlorhexidine, quinacrine dihydrochloride and chloroquine diphosphate (Sigma Chemical Co., St. Louis, MO); sucrose, ammonium sulfate, perchloric acid, isoamylalcohol (Ajax Chemicals, Australia); toluene (May & Baker, Australia); *S*-adenosyl-L-(^{14}C-N^{α}-methyl) methionine (SAM) (55 mCi/mmol) (New England Nuclear, Boston, MA); alcuronium dichloride (Hoffman-La Roche, Basel); amodiaquine hydrochloride (Parke Davis, Belgium).

(b) *Methods*

Determination of HMT inhibitory activity. The activity of HMT was determined by measuring the formation of ^{14}C-N^{α}-methylhistamine according to a modification of the method of Shaff and Beaven [8]. The assays were carried out in triplicate on 20-μl samples, and the incubation procedure performed at 25° for 2 hr.

The compounds, whose influence on HMT activity was tested, were prepared in 0.05 M potassium phosphate buffer pH 7.9, so that 0.0001–20 nmol compound in 10 μl was preincubated with HMT and SAM for 10 min before the addition of 3.5 ng of histamine in the same buffer. Blanks contained enzyme and SAM in 0.5 M potassium phosphate buffer pH 7.9 and standards over the range of 0–3.5 ng of histamine were prepared.

Counting of an aliquot of the organic extract was

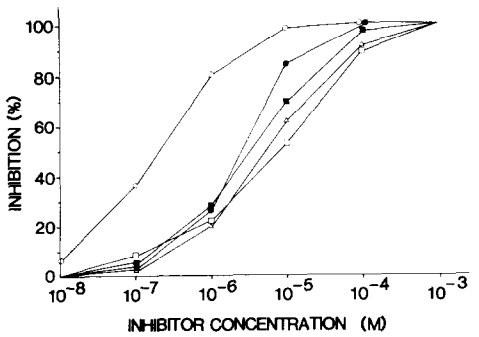


Fig. 1. Inhibition of HMT activity from rat kidney by 3 antimalarial drugs, chlorhexidine and alcuronium. Key to symbols: ○, amodiaquine; ●, quinacrine; □, chloroquine; ■, chlorhexidine; △, alcuronium.

Table 1. Inhibition of rat kidney HMT by three antimalarial drugs, the antiseptic agent chlorhexidine and the neuromuscular blocker alcuronium

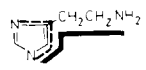
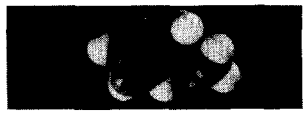
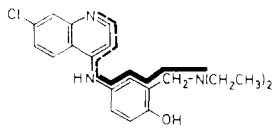
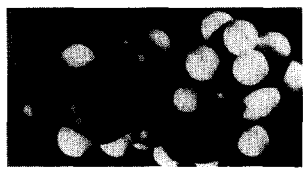
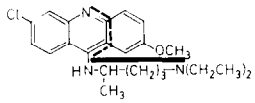
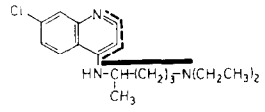
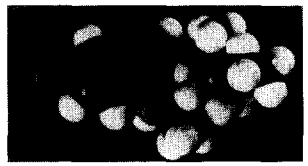
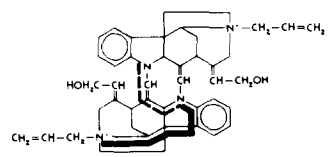
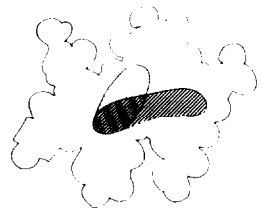
Compound	% Inhibition of HMT activity at a concentration (M) of					
	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
Amodiaquine	100	100	98	80	36	6
Quinacrine	100	100	84	26	3	0
Chloroquine	100	89	52	21	8	0
Chlorhexidine	100	97	69	27	4	0
Alcuronium	100	91	61	20	2	0

performed in a liquid scintillation spectrometer (Tri-Carb 2425, Packard). The mean blank value for the assay was 65 ± 2 cpm, while uninhibited histamine solution gave 1445 ± 22 cpm.

RESULTS AND DISCUSSION

Three antimalarial drugs amodiaquine, quinacrine and chloroquine were compared side-by-side with the antiseptic agent chlorhexidine and the NMB alcuronium for their capacity to competitively inhibit rat kidney HMT activity *in vitro* (Fig. 1 and Table 1). Amodiaquine proved to be significantly more potent an inhibitor than the other compounds tested over the concentration range 10^{-3}–10^{-8} M and produced 80% inhibition at 10^{-6} M, whilst the next best inhibitor at this concentration, chlorhexidine, produced 27% inhibition. Our results thus confirm that amodiquine is the most potent *in vitro* inhibitor of HMT known. Chlorhexidine was found to be more active than both chloroquine and alcuronium particularly in the concentration range 10^{-3}–10^{-6} M.

Table 2 Competitive inhibitors of rat kidney HMT showing structural similarities to histamine .

Compound	Structure	Space-filling model	Outline of model with structural similarities shaded
Histamine			
Amodiaquine			
Quinacrine			
Chloroquine			
Alcuronium			

The broken and unbroken lines on the structures correspond to the dotted and hatched regions respectively on the outlines of the models

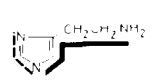
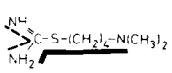
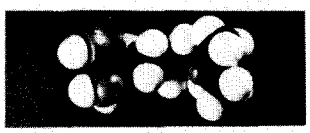
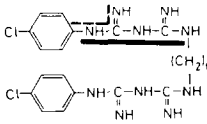
In a previous study [3], the three antimalarials were compared side-by-side for inhibitor activity with guinea-pig brain HMT. The inhibition observed with rat kidney HMT appears to parallel the results found in this study, i.e. amodiaquine is a better inhibitor than quinacrine which is better than chloroquine. The inhibitory potencies observed with rat kidney HMT, however, are less than that found with guinea-pig brain HMT.

Beaven and Shaff [5] have noted that the diversity of chemical structures of competitive inhibitors of HMT for its substrate histamine makes it difficult to visualise the structural features responsible for the inhibition. As an explanation for the observed inhibitions, they simply proposed that these compounds act by binding reversibly to, or near, the active site of the enzyme. Amodiaquine, chloroquine and quinacrine are known to be competitive inhibitors of HMT with respect to histamine [3] and our studies have shown that alcuronium and chlorhexidine are also competitive inhibitors of the enzyme. Demonstration of inhibitory activity by chlorhexidine and alcuronium further demonstrates the structural diversity found amongst HMT inhibitors but, even with the apparent diversity, it seemed likely that subtle structural similarities between histamine and the different inhibitors would be found. Examination of the structural formulae and space-filling models of amodiaquine, quinacrine and chloroquine (Table 2) and comparison of these with a model of histamine did, in fact, reveal some close similarities. Both histamine and the antimalarial drugs contain a sequence of two N atoms separated by four C atoms (outlined by unbroken line) and another sequence of two N atoms separated by two or three C atoms (outlined by broken line). With the NMB alcuronium, a sequence of two N atoms separated by five C atoms and another sequence of two N atoms separated by three C atoms is again similar to the sequences observed with histamine (Table 2). The space-filling models revealed that the observed sequences are probably accessible for enzyme binding and are conformationally similar.

Structural similarities can also be seen with another class of potent HMT inhibitors represented by chlorhexidine (Table 3). With this compound, the repeating structure —NH—C=N (outlined by broken line) can be seen on one side of the histamine molecule encompassing both Ns of the imidazole ring and another sequence of two N atoms separated by two C atoms and another N atom (unbroken line). In the CPK model of chlorhexidine, the relationship between the three N atoms exposed on one side of the molecule are strikingly similar to that of histamine (Table 3). Chlorhexidine is structurally very similar to two molecules of the antimalarial drug chlorguanide linked together and is considerably more potent as an inhibitor of HMT activity than chlorguanide [3]. A likely explanation for this potency difference is that compared to histamine, chlorhexidine has twice as many sites capable of binding to the active site on the enzyme. The potent guinea-pig brain HMT inhibitor SKF 91488 has structural features in common with histamine and chlorhexidine, namely a NH—C=N unit (broken line in Table 3) and another sequence of two N atoms separated by five C atoms and an S atom (unbroken line). The space-filling model, however, reveals that this compound is conformationally similar to histamine as a result of the S atom being on the other face of the molecule. Thus, all five compounds tested here, and SKF 91488, share a common structural feature(s) with histamine.

In our previous study where we investigated the inhibitory effect of NMB drugs on the activity of HMT *in vitro* [4], we found that NMB drugs with rigid structures, i.e. alcuronium, *d*-tubocurarine and pancuronium, were better inhibitors than the straight chain drugs such as succinylcholine and decamethonium. This finding suggested that the former compounds show a greater degree of complementarity towards the inhibitory sites of the enzyme. This observation is borne out in the present study where amodiaquine, which contains a relatively bulky aromatic substituent and hence provides less freedom to rotate around the secondary N atom,

Table 3. Competitive inhibitors of rat kidney HMT showing structural similarities to histamine .

Compound	Structure	Space-filling model	Outline of model with structural similarities shaded
Histamine			
SKF 91488			
Chlorhexidine			

The CPK model and structural outline show only one half of the chlorhexidine molecule.

The broken and unbroken lines on the structures correspond to the speckled and hatched regions respectively on the outlines of the models.

inhibited HMT activity better than quinacrine and chloroquine which have an alkyl-amine substituent instead of an aromatic-amine substituent (Table 2).

Electronic considerations may provide another explanation for the very potent inhibition exhibited by amodiaquine. Firstly, the aromatic substituent is an electron rich source which may enhance the binding of the drug to the active site of the enzyme if there is an electron deficient region at, or near, the binding site. Secondly, the hydrogen on the phenolic group (Table 2) is capable of forming H bonds with electron donating groups which may be present at, or near, the active site. The two considerations of steric and electrostatic interactions resulting from the presence of an aromatic group on amodiaquine would suggest that this region of the molecule is important for enzyme recognition.

As more compounds are found to inhibit HMT activity, it will be interesting to see whether they also show the structural features and conformational similarities with histamine identified here.

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