[^{14}C]Guanidine uptake into purified synaptosomes can be used as a model for measuring sodium flux as demonstrated by Pauwels *et al.* [11]. In the present study, veratridine (60 μM)-stimulated guanidine uptake into mouse cerebrocortical synaptosomes was measured over a time period of 40 sec during which the uptake was still linear with time (data not shown). Tetracaine and cocaine inhibited guanidine uptake with IC_{50} values of 25 and 140 μM respectively (Fig. 3B). Thus, tetracaine is more potent than cocaine in inhibiting both veratridine-stimulated guanidine flux in synaptosomes and BTX-stimulated inositide hydrolysis in slices, consonant with the idea that the two effects are related. Tetracaine has been shown to be more potent than cocaine also in other sodium channel assays: there is an 8-fold difference in potency between the drugs for inhibition of BTX-induced depolarization in synaptosomes [12], a 15-fold [12] or 16-fold [6] difference for inhibition of [^{3}H]BTX-B binding, and an 8-fold difference for acceleration of dissociation of [^{3}H]BTX-B binding from synaptosomes in the presence of aconitine [6]. In the inositide assay, tetracaine was more potent than cocaine as indicated by the shift to the right in the BTX activation curve in the presence of 100 μM tetracaine as compared with 300 μM cocaine (Fig. 2) and by the 50-fold difference in inhibiting potency after stimulation with 0.05 μM BTX (Fig. 3A). The high IC_{50} value of 100 μM for cocaine in inhibiting BTX (0.05 μM)-elicited inositide turnover (Fig. 3) makes the pharmacological relevance of this effect doubtful. However, the IC_{50} value will depend on the concentration of activator used, and it should be recalled that levels of cocaine in the brain of cocaine addicts can be expected to be as high as 2–10 μg/ml (7–33 μM) after a dose of cocaine normally administered intranasally, intravenously, or intrapulmonarily (for citations see Ref. 6). Such levels, if present at sodium channels, can be expected to inhibit sodium uptake and to affect the input of sodium channel activity into the phosphatidylinositol system. The present results do not negate the possibility that blockade of monoamine uptake by cocaine could lead to increased inositide hydrolysis in neurons receiving monoaminergic innervation. Perhaps a superfused and stimulated slice preparation could be used to demonstrate an inositol response as a result of monoamine uptake inhibition. The present results, however, do show that the increase in inositide hydrolysis induced by a sodium channel activator can be counteracted by concentrations of cocaine that inhibit the flux of guanidine through activated sodium channels. In addition, the correlation between the potencies of cocaine and tetracaine in inhibiting BTX-elicited inositide hydrolysis and those in inhibiting guanidine flux suggests that the former effect of cocaine is mediated by inhibition of sodium flux.

In conclusion, this study demonstrates that cocaine can interact with the phosphoinositide system at concentrations that inhibit sodium flux. The stimulation of inositide hydrolysis by BTX, aconitine, and ScVenom in mouse cerebrocortical slices was reduced by the presence of 5 μM TTX. Cocaine and tetracaine shifted the dose–response curves of BTX in stimulating inositide hydrolysis to the right. In addition, cocaine and tetracaine inhibited BTX (0.05 μM)-elicited inositide hydrolysis with IC_{50} values of approximately 100 and 2 μM respectively. Finally, cocaine and tetracaine inhibited veratridine (60 μM)-stimulated [^{14}C]guanidine uptake into synaptosomes with IC_{50} values of approximately 140 and 25 μM respectively.

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Scavenging of hypochlorous acid by tetracycline, rifampicin and some other antibiotics: a possible antioxidant action of rifampicin and tetracycline?

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Antibiotics are widely used in the treatment and prophylaxis of acute and chronic infections. As well as their antibacterial actions, they have multiple effects on the immune system (reviewed in [1]). For example, several antibiotics have been reported to decrease bacterial killing by activated phagocytes, and the possibility that antibiotics might interfere with phagocyte killing mechanisms has been raised [1–3]. Activated phagocytes produce [4, 5] superoxide radical (O_2^-), hydrogen peroxide (H_2O_2) and, possibly, the highly-reactive hydroxyl radical (·OH), all of which are important agents in bacterial killing and in the damage to surrounding tissues that can be produced by activated phagocytic cells

[4–6]. Miyachi *et al.* [7] reported that several antibiotics decrease oxygen radical production by neutrophils, although they claimed that antibiotics themselves are poor scavengers of oxygen radicals.

Activated neutrophils contain the enzyme myeloperoxidase, some of which is released during phagocytosis. Myeloperoxidase uses H_2O_2 to oxidise Cl^- ions into a powerful oxidant that has been identified as hypochlorous acid, HOCl [4, 6]. HOCl plays some part in the killing of fungi and bacteria by neutrophils [4], but it can also do considerable tissue damage at sites of inflammation because of its high reactivity [6]. Perhaps the most important target of HOCl is α_1-antiproteinase (α_1AP) the inactivation of which by HOCl permits uncontrolled proteolytic activity, especially that of elastase [6, 8].

Several anti-inflammatory drugs react with HOCl [9], but in few cases is the reaction fast enough, at the drug concentrations that are achieved *in vivo*, to protect α_1AP against HOCl attack [10]. In the present paper, we have investigated the ability of several antibiotics in current clinical use to scavenge HOCl, in order to establish whether they might be able to do this under physiological conditions.

Materials and methods

Reagents. Porcine pancreatic elastase and sodium hypochlorite were from BDH Chemicals Ltd. Poole, Dorset, U.K. Antibiotics, α_1-antiproteinase and other reagents were of the highest quality available from Sigma Chemical Corp. HOCl was obtained immediately before use by adjusting sodium hypochlorite (Na^+OCl^-) to pH 6.2 with dilute H_2SO_4 [11].

Assays. Elastase and α_1-antiproteinase were assayed at pH 7.4 as described in [11]. α_1-Antiproteinase (final concentration 1.0 mg/ml) was, unless otherwise stated, mixed with the final concentration of antibiotic quoted before adding HOCl (75 μM final concentration). The final reaction mixture (35.1 μl volume) also contained buffer (140 mM NaCl, 16 mM Na_2HPO_4, 2.9 mM KH_2PO_4, pH 7.4) and it was incubated at 25° for 60 min. Then 3 ml of the same buffer was added, followed by elastase. After further incubation for 30 min, elastase substrate was added and the elastase activity remaining was assayed as a rise in absorbance at 410 nm [11]. Results are expressed as % of maximum elastase activity, where 100% corresponded to a ΔA_{410} of 0.056 min^{-1}. Results are the averages of duplicate determinations that varied by <5%. In some experiments, the HOCl and antibiotic were pre-incubated for 5 min before adding α_1AP, then incubated for a further 60 min before adding buffer and elastase, as described above. Where necessary, stock solutions of antibiotics were adjusted to pH 7.4. Addition of the concentrations of HOCl used in these experiments did not alter the pH of reaction mixtures, which was kept at 7.4 throughout.

Results

None of the antibiotics used in this study, at the final concentrations present in the reaction mixtures, had any effect on elastase activity. α_1-Antiproteinase inhibits elastase, and a final concentration of 1.0 mg/ml, close to the normal human serum α_1-antiproteinase concentration of 1.3 mg/ml [12], was used to inhibit elastase completely (Table 1, columns A and B, first line). Control experiments showed that none of the antibiotics affected the ability of α_1-antiproteinase to inhibit elastase.

HOCl, the oxidant produced by the action of myeloperoxidase [6] in activated neutrophils, inactivates α_1-antiproteinase and allows expression of elastase activity (Table 1, columns A and B, second line). The concentration of HOCl used in these studies (75 μM) is within the range of concentrations known to be achieved adjacent to activated neutrophils [13, 14]. The effect of including various antibiotics in the reaction mixture together with α_1-antiproteinase just before adding HOCl was examined (Table 1, column A). If an antibiotic can scavenge the added HOCl quickly enough to protect α_1-antiproteinase, the elastase-inhibitory capacity of this protein will be preserved and the final elastase activity measured will decrease. Table 1 (column A) shows that erythromycin, rifamycin SV and chloramphenicol, tested at concentrations up to 1 mM, were unable to protect α_1AP against attack by HOCl. However, penicillin G, tetracycline, rifampicin and streptomycin offered significant protection to α_1AP. The effects of tetracycline and rifampicin were especially marked (>90% protection against 75 μM HOCl at 400 μM antibiotic concentration).

Table 1 (column B) shows what happened when the antibiotics were pre-incubated with the HOCl for 5 min before adding α_1AP. In all cases, the elastase-inhibitory capacity of the added α_1AP remained, i.e. there was no HOCl left to inactivate α_1AP. Hence, all the antibiotics are capable of reacting with HOCl when they are preincubated with it, but only in the cases of penicillin G, tetracycline, rifampicin and streptomycin are the reactions fast enough to protect α_1AP present simultaneously in the reaction mixture when HOCl is added.

Discussion

HOCl is a powerful oxidant that can attack a wide range of biomolecules [9–11, 15, 16]. It is produced by the enzyme myeloperoxidase in activated neutrophils. In these cells, a considerable amount of myeloperoxidase enters the phagocytic vacuole, and will produce HOCl there [6]. Some myeloperoxidase is released from the neutrophils, and produces HOCl external to them. For example, 5×10^6 activated human neutrophils were reported to produce 88.3 ± 23.6 nmol of HOCl in 1 ml at 22° [13], which corresponds to concentrations of 60–100 μM. At sites of inflammation *in vivo*, concentrations will be less than this because of scavenging of HOCl by various biomolecules [11], but it seemed reasonable to use 75 μM HOCl in our experiments. The most important extracellular target attacked by HOCl is probably α_1AP [6]. Thus the scavenging of extracellular HOCl by a drug seems unlikely to be physiologically significant unless, at the concentration of drug present *in vivo*, scavenging is rapid enough to protect α_1-antiproteinase against attack by HOCl [10]. HOCl is capable of reacting with all the antibiotics tested in this study (Table 1, column B), in that they remove HOCl when they are incubated together with it at pH 7.4. However, only in the case of four antibiotics does it seem that the reaction might be fast enough to contribute to their biological activities. For penicillin, effective serum therapeutic concentrations are 3–5 μg/ml (approximately 13 μM [17]) whereas scavenging of 75 μM HOCl requires over a ten-fold molar excess of penicillin (1000 μM; Table 1). Hence HOCl scavenging seems unlikely to contribute to the effects of penicillin at normal doses. A similar conclusion may be valid in the case of streptomycin, for which the peak serum level giving maximum therapeutic effect is 25–30 μg/ml, or 51.6 μM [17], whereas 600 μM streptomycin is required to scavenge 75 μM HOCl effectively. However, both rifampicin and tetracycline are effective in scavenging 75 μM HOCl at concentrations of around 200 μM (Table 1). It thus seems that HOCl scavenging might contribute to the physiological effects of rifampicin and tetracycline. For example, Greenwald *et al.* [18] reviewed data showing that tetracyclines diminish collagenase activity at sites of inflammation. Since HOCl activates latent collagenase [6], the ability of tetracyclines to scavenge HOCl might contribute to a decrease in collagenase activity.

HOCl generated in the phagocytic vacuole contributes to the anti-bacterial activity of neutrophils, and is especially important in their anti-fungal activity [19, 20]. Both tetracycline and rifampicin are concentrated into phagocytes [3], although how much, if any, enters the phagocytic

Table 1. Effect of antibiotics on inactivation of α_1AP by hypochlorous acid, HOCl

Antibiotic added	Concentration (mM)	Elastase activity (% maximum rate) Column A HOCl added to α_1AP and antibiotic together	Column B HOCl and antibiotic preincubated before adding to α_1AP
None (HOCl omitted)	—	0	0
None	—	91	94
Penicillin G	0.2	94	1
(benzylpenicillin,	0.4	77	1
sodium salt)	0.6	52	<1
	0.8	37	0
	1.0	24	0
Erythromycin	1.0	100	1
Tetracycline	0.1	82	<1
(hydrochloride)	0.2	49	0
	0.4	9	0
	0.6	5	0
	0.8	1	0
Rifampicin	0.05	97	<1
	0.1	67	<1
	0.2	23	0
	0.4	5	0
	0.6	4	0
	0.8	2	0
Rifamycin SV (sodium salt)	1.0	102	<1
Chloramphenicol	1.0	100	23
Streptomycin	0.1	99	<1
(sulphate)	0.2	87	<1
	0.4	46	0
	0.6	15	0
	0.8	2	0
	1.0	3	0

vacuole is unknown and would be very difficult to measure. It is interesting to note that both tetracycline [1] and rifampicin [3] have been reported to decrease microbial killing by neutrophils, whereas penicillin does not [3]; observations which suggest that HOCl scavenging by tetracycline and rifampicin accounts for these effects.

It thus seems that, by scavenging HOCl, tetracyclines and rifampicin could have antioxidant actions at sites of inflammation. They can also have pro-oxidant actions. In the presence of light, tetracyclines generate singlet O_2 and O_2^- [21–24] and complexes of tetracyclines with transition metal ions generate ·OH [25].

There is an alternative explanation for the inability of erythromycin, rifamycin SV, and chloramphenicol to protect α_1AP against inactivation by HOCl (Table 1, column A). Some compounds, such as taurine, react with HOCl to produce a product that can itself inactivate α_1AP [6]. This could explain the failure of the above antibiotics to protect, if it is assumed that the reactive product formed could not survive 5 minutes incubation (or else the drugs could not protect on preincubation; Table 1 [column B]). However, such an alternative explanation does not alter the conclusion that reaction of HOCl with erythromycin, rifamycin SV or chloramphenicol cannot achieve protection of α_1AP against inactivation by HOCl *in vivo*.

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