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Characterization of 5-hydroxytryptamine receptors mediating mucosal secretion in guinea-pig ileum

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1 The receptor subtypes through which 5-hydroxytryptamine (5-HT) increases electrolyte secretion across the mucosa of guinea-pig ileum were studied.

2 Flat sheep preparations of guinea-pig mucosa plus submucosa were placed in Ussing chambers and the short circuit current (I_{SC}), an index of net electrogenic electrolyte transport across the mucosa, was measured under voltage clamp conditions.

3 Low concentrations of 5-HT (10–300 nM) evoked monophasic increases in I_{SC} which were significantly reduced by hyoscine (100 nM), tetrodotoxin (TTX, 300 nM) and the 5-HT_2 receptor antagonist, ketanserin (3–300 nM).

4 Higher concentrations of 5-HT (1–10 μM) produced biphasic responses which were reduced by hyoscine (100 nM), TTX (300 nM), ketanserin (3–300 nM) and also by the 5-HT_3 receptor antagonists, granisetron (1 μM) and ICS 205-930 (100 nM).

5 2-Methyl-5-HT (1–100 μM) and α-methyl-5-HT (30 nM–30 μM), agonists at 5-HT_3 and 5-HT_2 receptors respectively, also evoked I_{SC} increases. These responses were reduced by hyoscine (100 nM) and abolished by TTX (300 nM) and the respective receptor antagonists, granisetron (1 μM) and ketanserin (30 nM).

6 The 5-HT_4 receptor antagonist, SDZ 205-557 (300 nM) had no effect on the response to 5-HT.

7 The TTX-resistant response to 5-HT was not affected by 5-$HT_{2,3\text{ or }4}$ receptor antagonists.

8 These results indicate that 5-HT mediates secretion partly by an action on 5-HT_3 receptors located on cholinergic and noncholinergic secretomotor neurones, partly by an action on higher affinity '5-HT_2-like' receptors predominantly on noncholinergic neurones, and partly by a direct action on the epithelium.

Keywords: 5-Hydroxytryptamine; mucosal transport; secretomotor neurones; 5-HT_2 receptors; 5-HT_3 receptors; intestine

Introduction

5-Hydroxytryptamine (5-HT) produces a wide variety of effects on central and peripheral neurones. This variety is due in part to the presence of a number of 5-HT receptor subtypes (Bradley *et al.*, 1986; Watson & Girdlestone, 1993). 5-HT promotes mucosal secretion of water and electrolytes in the intestine of many species, including the rat (Hardcastle *et al.*, 1981), guinea-pig (Cooke & Carey, 1985) and rabbit (Donowitz *et al.*, 1977). In the guinea-pig ileum this secretory response appears to be mediated via neurones in the submucous plexus (Keast *et al.*, 1985; Cooke & Carey, 1985). Flux chamber experiments using isolated mucosa plus submucosa preparations have shown that 5-HT evokes a biphasic increase in short circuit current (I_{SC}) which has been attributed to an increase in chloride secretion (Cooke & Carey, 1985).

Multiple sites exist in the gut for the potential action of 5-HT. Receptors for the amine have been localized on cell bodies of neurones in the submucous plexus (Hirst & Silinksy, 1975; Surprenant & Crist, 1988). Pharmacological data suggest these receptors exist on both cholinergic and noncholinergic secretomotor neurones (Keast *et al.*, 1985). 5-HT may also have a small direct secremotor effect on the mucosal epithelium (Zimmerman & Binder, 1984), although ligand binding studies have been unable to locate epithelial receptors for 5-HT (Gaginella *et al.*, 1983).

Characterization of the receptor subtypes mediating secretion has been limited by a lack of selective pharmacological tools. Hendriks and colleagues (1989) found evidence for 5-HT_3 receptors on cholinergic secretomotor neurones, but were unable to classify the receptors on the noncholinergic neurones. However, the recent development of more selective 5-HT drugs has led us to re-investigate this problem.

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Methods

Tissue preparation

Guinea-pigs of either sex weighing between 150 and 300 g were stunned by a blow to the head and then killed by severing the carotid arteries and spinal cord. A 10 cm segment of ileum was taken approximately 10–30 cm from the ileo-caecal junction and placed in physiological saline. The segment was opened along the mesenteric border and pinned flat with the mucosal surface down. The longitudinal and circular muscle layers and myenteric plexus were removed and the remaining mucosa plus submucous plexus preparation was divided into flat 1 cm^2 sheets and mounted in Ussing flux chambers (window area 0.38 cm^2). Four preparations were made from adjacent segments of intestine from each animal. One served as an internal control (i.e. for construction of a concentration-effect curve in the absence of the drugs being tested), the other three were used to test different combinations of drugs. In the data outlined below, the number of preparations used refers to the number of animals.

The tissues were bathed on both sides with physiological saline at 37°C containing (in mM): NaCl 118, $NaHCO_3$ 25, KCl 4.8, $CaCl_2$ 2.5, $MgSO_4$ 1.2, NaH_2PO_4 1.0, and gassed with 95% O_2, 5% CO_2. Glucose (11 mM) was added to the serosal bathing solution and mannitol (11 mM) to the mucosal side to prevent coupled sodium absorption of glucose which may have affected the measurement of secretion.

Electrical measurements

The potential difference across the tissue was measured by Krebs-agar bridges mounted on either side of the preparation and connected to calomel reference electrodes. This potential difference was clamped to zero, with a suitable compensation for fluid resistance, by a voltage clamp (Physiological Instruments Voltage-Current Clamp model VCC600); the current required to do this, termed the short-circuit current (I_{SC}; Ussing & Zerahn, 1950), was recorded as an index of net electrogenic electrolyte transport. The responses were recorded directly on to a CODAS data acquisition programme (DATAQ).

Experimental protocol

The tissue was left to equilibrate for at least 1 h or until stable baseline I_{SC} responses were achieved. Baseline I_{SC} values were similar to those described in previous studies using identical Ussing chambers (Keast *et al.*, 1985; Hendriks *et al.*, 1989). All drugs were added to the serosal bathing solution. Repeated doses of 5-HT (1 μM) were added to the baths at 30 min intervals (up to 4 repetitions) until it was ascertained that the response to this concentration of 5-HT was consistent and could serve as a control. This concentration was chosen because it had previously been shown to evoke a submaximal response without desensitizing the tissue using a similar protocol (Hendriks *et al.*, 1989). No significant differences were observed in control I_{SC} values either within or between groups of experiments.

Non-cumulative concentration-effect curves were performed by adding increasing concentrations of agonists at 30 min intervals. Agonists were left in contact with the tissue for 2.5 min before washout. In some experiments, and in previous studies (Keast *et al.*, 1985; Hendriks *et al.*, 1989), a small but significant increase in the tissue response to 5-HT was observed during the time (up to 5 h) needed for these experiments. When used, antagonists were added 15 min prior to each addition of an agonist.

Analysis of results

The maximum amplitudes of the I_{SC} responses to the agonists were expressed as percentages of the amplitude of the initial stable 5-HT 1 μM control response. When the response was biphasic (see Figure 1), the amplitude of the larger phase was measured although this sometimes changed from the first to the second phase with drug treatment. Although this may have introduced some distortions with high concentrations of 5-HT, most experiments were carried out under conditions where the responses were monophasic. Concentration-effect curves for the agonists in the presence and absence of antagonists were presented graphically and compared by two-way analysis of variance.

Drugs

The following drugs were used: 5-hydroxytryptamine creatinine sulphate complex (5-HT), hyoscine hydrobromide, tetrodotoxin (TTX; Sigma); 2-methyl-5-hydroxytryptamine maleate, α-methyl-5-hydroxytryptamine maleate, cyproheptadine hydrochloride, ketanserin (+)-tartrate (Research Biochemicals Incorporated); ICS 205-930 (tropisetron), granisetron (BRL 43694, SmithKline Beecham); SDZ 205-557 (2-methoxy-4-amino-5-chloro-benzoic acid 2-(diethylamino) ethyl ester; gift from SmithKline Beecham). All drugs were dissolved in physiological saline except ICS 205-930 which was dissolved in 0.1 M hydrochloric acid. The vehicle was shown to have no effect on secretion or the response to drugs.

Results

Effects of 5-HT on I_{SC}

As previously described by Keast *et al.* (1985), 5-HT produced a concentration-dependent rise in I_{SC} with a maximum increase of 97.5 ± 2.6 μA cm^{-2} and an apparent EC_{50} of 0.7 μM ($n = 8$). Low concentrations of 5-HT (10–300 nM) evoked a monophasic increase in I_{SC} whereas higher concentrations (1–30 μM) produced a biphasic response composed of an initial rapid transient rise in I_{SC}, followed by a slower more maintained phase that was similar to the response evoked by lower 5-HT concentrations (Figure 1).

Concentration-effect curves were established for 5-HT, 2-methyl-5-HT (a relatively selective 5-HT_3 receptor agonist; Mawe *et al.*, 1986) and α-methyl-5-HT (an agonist with some selectivity for 5-HT_2 receptors; Richardson *et al.*, 1985). The effects of various selective receptor antagonists on these concentration-effect relationships were investigated to determine the roles of the different receptor subtypes.

Effect of hyoscine on the response to 5-HT

The muscarinic receptor antagonist, hyoscine (100 nM), abolished the first phase of the response to high concentrations of 5-HT (>300 nM), and reduced, but did not abolish the second component (see also Keast *et al.*, 1985; Hendriks *et al.*, 1989). The response evoked by lower 5-HT concentrations was also decreased. Overall, the effect of hyoscine on the I_{SC} response was significant ($P < 0.005$, $n = 4$, Figure 2a).

5-HT_3 receptors

The selective 5-HT_3 receptor antagonist, granisetron (1 μM; Sanger & Nelson, 1989) decreased the amplitudes of both phases of the I_{SC} response evoked by high concentrations of 5-HT (>300 nM); the decrease in the maximum amplitude of the response was significant ($P < 0.005$, $n = 4$). The second phase was apparently reduced ($P < 0.05$, $n = 4$), but this may have been solely due to the decrease in the overlapping first phase. The response to lower concentrations of 5-HT was unaffected by granisetron (Figures 1 and 2a).

2-Methyl-5-HT had no effect on secretion at concentrations below 1 μM, but evoked a biphasic increase in I_{SC} at higher concentrations (Figure 1), with an EC_{50} of 6.6 μM ($n = 4$). This response was significantly decreased by granisetron (1 μM; $P < 0.0001$, $n = 4$), and abolished by TTX (300 nM; $P < 0.0001$, $n = 4$; Figure 2b). Hyoscine (100 nM) abolished the first phase of the response to 2-methyl-5-HT but had little effect on the second component. The maximum amplitude of the I_{SC} response was reduced by an average of 64% (Figures 1 and 2b; $P < 0.0001$, $n = 4$). The initial com-

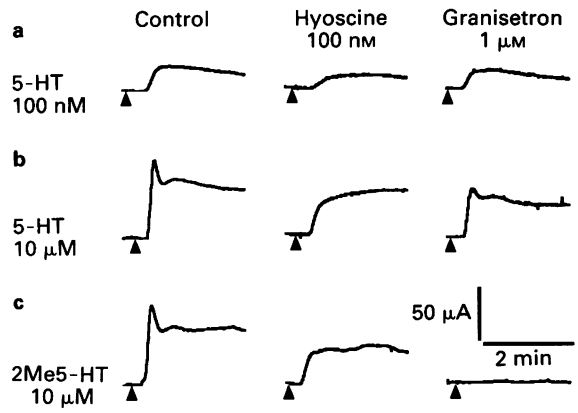

Figure 1 Representative records illustrating control I_{SC} responses to 5-hydroxytryptamine (5-HT) and 2-methyl-5-HT, and responses in the presence of hyoscine (1 μM) and granisetron (1 μM): (a) 5-HT 100 nM; (b) 5-HT 10 μM; (c) 2-methyl-5-HT 10 μM; (▲) indicate the times of addition of agonists.

ponent of the response to 2-methyl-5-HT, like that to high concentrations of 5-HT, appears to be due to the action of acetylcholine on muscarinic receptors on the mucosal epithelium (Keast *et al.*, 1985). ICS 205-930, an antagonist at both 5-HT_3 and 5-HT_4 receptors, also reduced the response evoked by 2-methyl-5-HT (data not shown) when used at 100 nM, a concentration expected to block only 5-HT_3 receptors (Craig & Clarke, 1990).

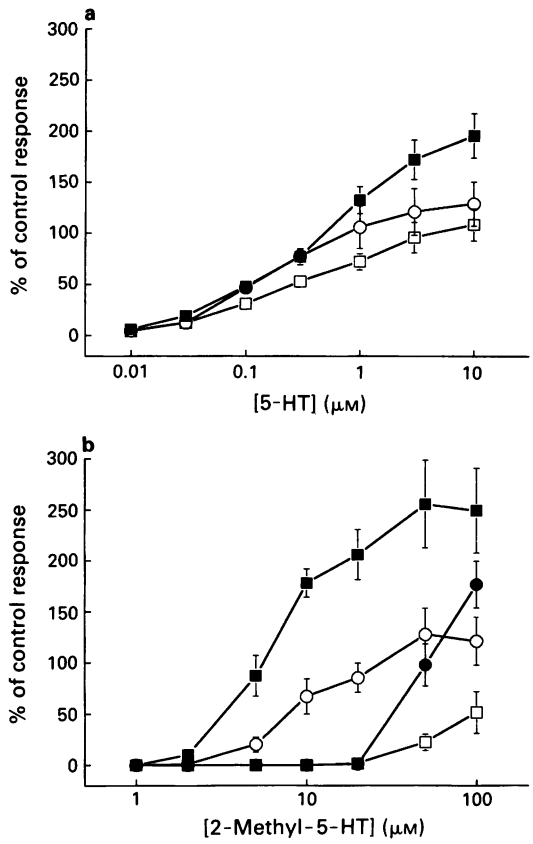

Figure 2 Concentration-effect curves for (a) 5-hydroxytryptamine (5-HT) alone (■) and in the presence of granisetron (1 μM, ○) or hyoscine (100 nM, □); and (b) 2-methyl-5-HT alone (■) and in the presence of granisetron (1 μM, ○), hyoscine (100 nM, □) or tetrodotoxin (300 nM, ●). Results were expressed as mean ± s.e.mean; $n = 4$.

5-HT_2 receptors

The 5-HT_2 receptor antagonist, ketanserin (3–300 nM), produced a significant depression of the responses evoked by all but the highest concentrations of 5-HT ($P < 0.05$, $n = 5$, Figure 3). It did not appear to abolish either component of the biphasic response to 5-HT. The rightward shift in the concentration-effect curve was similar in magnitude in the presence of three concentrations of ketanserin tested (3, 30 and 300 nM). This suggested that the effects of low concentrations of 5-HT might be mediated via 5-HT_2 receptors.

Results similar to those seen with ketanserin were obtained with cyproheptadine (100 nM) which depressed responses to all concentrations of 5-HT (not illustrated). However, this concentration of cyproheptadine also depressed responses to carbachol suggesting that its effects on high concentrations of 5-HT may have been mediated, at least in part, via an action on muscarinic receptors on the mucosa.

α-Methyl-5-HT, a predominantly 5-HT_2 receptor agonist, evoked a monophasic increase in I_{SC} with a similar time course to the second phase of the response to 5-HT. This response was almost completely abolished by ketanserin (30 nM; $P < 0.0001$; $n = 4$). In the presence of hyoscine (100 nM), the amplitude of the I_{SC} increase evoked by α-methyl-5-HT was reduced by approximately 30% (Figure 4). These experiments were performed in the presence of ICS 205-930 (3 μM) to block any residual effect of the agonist at 5-HT_3 or 5-HT_4 receptors.

5-HT_4 receptors

The 5-HT_4 receptor antagonist, SDZ 205-557 (300 nM) (Buchheit *et al.*, 1991), had no significant effect on the response to 5-HT ($n = 4$; data not shown). Furthermore, a substantial TTX-sensitive component of the response persisted even in the presence of 3 μM ICS 205-930, ($n = 4$; data not shown; see also Hendriks *et al.*, 1989) a concentration expected to block both 5-HT_3 and 5-HT_4 receptors (Craig & Clarke, 1990).

Effects of TTX on the 5-HT response

Addition of TTX (300 nM) to the serosal bathing solution decreased the baseline I_{SC} by approximately 30% ($n = 4$). In the presence of tetrodotoxin (300 nM), 5-HT evoked a slow, well maintained monophasic response. The TTX-resistant response could not be further reduced by granisetron (1 μM) or ketanserin (30 nM; Figure 5).

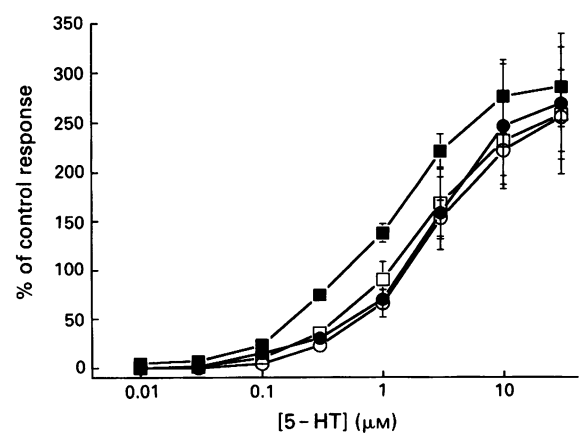

Figure 3 Effect of 5-HT_2 receptor antagonism: concentration-effect curves for 5-hydroxytryptamine (5-HT) in the absence (■) and presence of ketanserin (3 nM □, 30 nM ●, 300 nM ○). Results are expressed as mean ± s.e.mean; $n = 5$.

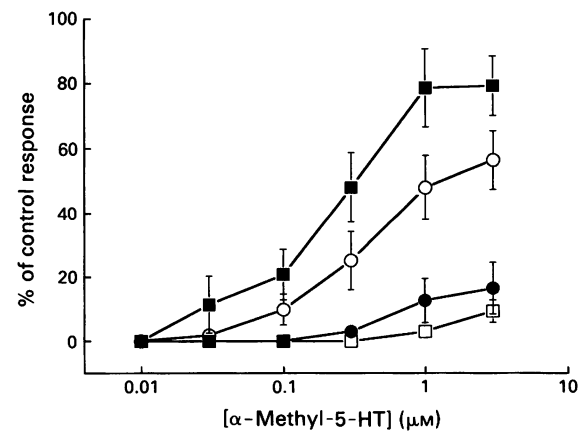

Figure 4 Concentration-effect curves for α-methyl-5-HT in the absence (■) and presence of ketanserin (30 nM, ●), hyoscine (1 μM, ○) or tetrodotoxin (300 nM, □). All curves were performed in the presence of ICS 205-930 (3 μM). Results are shown as means ± s.e.mean; $n = 4$.

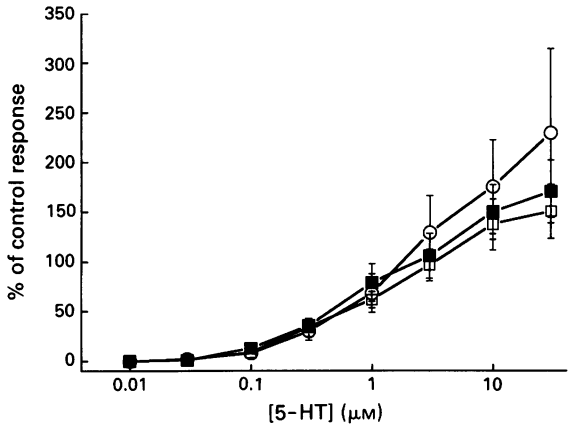

Figure 5 The effect of 5-HT_2 and 5-HT_3 receptor antagonists on the tetrodotoxin (TTX)-resistant response to 5-hydroxytryptamine (5-HT). Concentration-effect curves for 5-HT in the presence of TTX alone (300 nM, ■), TTX and ketanserin (30 nM, ○) or TTX and granisetron (1 μM, □). Results are expressed as means ± s.e.mean; $n = 4$.

Discussion

These results confirm that the primary effect of 5-HT in the promotion of intestinal secretion is to excite secretomotor neurones of the submucous plexus. The neurally mediated secretory response to 5-HT appears to be evoked via at least two distinct receptor subtypes.

Both cholinergic and noncholinergic secretomotor neurones were shown to be excited by 5-HT acting on 5-HT_3 receptors, a result consistent with those obtained by Surprenant & Crist (1988), who found that virtually all submucous neurones were depolarized by 5-HT acting on 5-HT_3 receptors. This contrasts with the conclusion of Hendriks *et al.* (1989), who found that the hyoscine-resistant component of the I_{SC} response to 5-HT (1 μM) was not further reduced by ICS 205-930, apparently demonstrating the presence of these receptors on cholinergic neurones only. However, it is possible that the noncholinergic secretomotor neurones were maximally stimulated via another receptor subtype, thereby masking the presence of 5-HT_3 receptors on these neurones. The use of the 5-HT_3 receptor agonist, 2-methyl-5-HT, in the present study has permitted a more direct test of the presence of this receptor.

The 5-HT_3 receptor antagonist, granisetron, decreased the response to 5-HT only at concentrations of 5-HT greater than 1 μM. This suggests that the 5-HT_3 receptor has a relatively low affinity for 5-HT, which is consistent with studies performed in other preparations (e.g. Buchheit *et al.*, 1985). 5-HT_3 receptor antagonists did not alter the 5-HT response. Thus it is unlikely that they play a significant role in the response of the preparation to 5-HT.

An intriguing result of the present study was the identification on some secretomotor neurones of a higher affinity 5-HT receptor which appears to belong to the '5-HT_2-like' receptor subtype. The response to 5-HT was substantially reduced by the 5-HT_2 antagonist, ketanserin. In addition, α-methyl-5-HT evoked a relatively large increase in I_{SC} which was almost completely blocked by ketanserin. Hysocine slightly reduced the response to α-methyl-5-HT. However, a substantial response persisted, indicating the existence of a population of '5-HT_2-like' receptors on both cholinergic and noncholinergic neurones. Figure 3 indicates that the effects of ketanserin were maximal with 3–30 nM of the antagonist. This suggests that the effects of 5-HT may be mediated via the 5-HT_{2A} receptor subtype (Humphrey *et al.*, 1993).

5-HT_2 receptors have been implicated in the secretory process in rat ileum, colon and jejunum (Hardcastle *et al.*, 1981; Moriarty *et al.*, 1987; Siriwardena *et al.*, 1991). Surprenant & Crist (1988) found that in addition to its effects at 5-HT_3 receptors, 5-HT evoked a slower, higher affinity depolarization in a subset of submucous neurones, which they were unable to attribute to a known receptor subtype. The high affinity receptor described by Surprenant & Crist (1988) may correspond to that detected in the present study, especially as they were able to detect only two excitatory effects of 5-HT on submucous neurones. However, Surprenant & Crist (1988) were unable to block the response to 5-HT with ketanserin, in direct contrast with the present study. Also, Hendriks *et al.* (1989) found no effect of cyproheptadine on the response to 5-HT that was resistant to ICS 205-930. Baird & Cuthbert (1987) found a small but non-significant effect of ketanserin on the increase in I_{SC} evoked by 5-HT. The present study suggests that the pharmacology of the electrophysiological effects of 5-HT on secretomotor neurones should be re-investigated.

A substantial response to 5-HT persisted in the presence of TTX which could not be attributed to an action at 5-HT_2, 5-HT_3 or 5-HT_4 receptors. As similar concentrations of TTX have been shown to block effectively action potential-dependent transmission from enteric neurones (Cooke & Carey, 1985), the residual response was probably due to a direct effect of 5-HT on the mucosal epithelium. Scott *et al.* (1992) examined the profile of a number of 5-HT_4 receptor agonists in the presence of TTX and concluded that a part of the TTX-resistant responses may be mediated via 5-HT_4 receptors. However, in the present study, the 5-HT_4 antagonist SDZ 205-557 (Buchheit *et al.*, 1991) had no effect on the response to 5-HT. Also, at concentrations of ICS 205-930 whch would be expected to block 5-HT_4 receptors there was no reduction in the response to low 5-HT concentrations. Thus it appears that this non-neural component of the 5-HT response may be mediated via an, as yet, unidentified receptor subtype. Experiments to characterize this receptor are currently underway.

In summary, the results provide evidence that mucosal secretion in response to 5-HT in the guinea-pig small intestine is mediated partly by an action on low affinity 5-HT_3 receptors present on both cholinergic and noncholinergic secretomotor neurones, partly by an action at high affinity '5-HT_2-like' receptors located predominantly on noncholinergic neurones, and partly by a direct action on the epithelium through receptors that have yet to be identified.

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