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STIMULATION OF THE ACTIVE TRANSPORT OF SEROTONIN INTO HUMAN PLATELETS BY HYDROGEN PEROXIDE

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Abstract—The effect of H_2O_2 on the active transport of serotonin (5-HT) by human platelets was investigated. Platelets were exposed to either a single dose of H_2O_2 or to H_2O_2 generated by the glucose/glucose oxidase or xanthine/xanthine oxidase enzyme systems. H_2O_2 (12.5 to 100 μM) produced a rapid, concentration-dependent and time-dependent increase in 5-HT transport which was maximal after a 2-min incubation and decreased with continued incubation. Catalase (1000 units) completely prevented H_2O_2-induced stimulation, and fluoxetine (1 μM) totally blocked 5-HT uptake into stimulated platelets. The glucose/glucose oxidase (3.12 to 100 milliunits) and the xanthine/xanthine oxidase generating systems produced a similar response to that of H_2O_2. In the xanthine/xanthine oxidase system, superoxide dismutase (250 units) failed to alter the stimulation, whereas catalase (1000 units) effectively prevented the response. The kinetics of 5-HT transport indicated that H_2O_2 treatment did not alter the K_m of 5-HT transport (K_m control = $1.0 \pm 0.2 \times 10^{-6}$ M vs K_m H_2O_2 = $1.1 \pm 0.1 \times 10^{-6}$ M) but markedly increased the maximal rate of 5-HT transport (V_{max} control = 131.4 ± 4.6 pmol/10^8 platelets/4 min vs V_{max} H_2O_2 = 206.7 ± 9.1 pmol/10^8 platelets/4 min). These data demonstrated that exposure of human platelets to H_2O_2 resulted in a stimulation of the active transport of 5-HT and suggested that H_2O_2 may function to regulate this process.

Hydroxide peroxide (H_2O_2)* is increasingly being implicated in tissue injury and human disease [1, 2] through its ability to oxidize sulfhydryl groups [3], induce chromosomal alterations and DNA breakage [4], inactivate transforming DNA [5], and produce general cellular toxicity [6]. Normal cellular metabolism produces small quantities of H_2O_2 which are detoxified effectively by catalase and glutathione peroxidase [7]. Most cells, including platelets, possess both of these defense enzymes [8, 9] which maintain the intracellular concentration of H_2O_2 below that required to produce injury. Cellular injury occurs when the concentration of H_2O_2 exceeds the inactivation capacity or escapes these intracellular defense enzymes or when H_2O_2 is present extracellularly. Such increased production of H_2O_2 can occur as a result of phagocytic cell activation [10], ischemia-reperfusion [11] or through redox cycling of drugs and environmental toxicants [12], all of which can result in circulating platelets being exposed to this oxidant.

Human platelets exposed to H_2O_2 have been shown to have altered morphology [13], biochemistry [14–16] and function [17]. For example, platelet aggregation was impaired [13, 17] and the metabolic pool of ATP was reduced irreversibly [14, 15] by concentrations of H_2O_2 similar to those generated by activated granulocytes [18]. Since platelet aggregation is energy-consuming and requires a threshold level of metabolic ATP, it has been suggested that its impairment may result from H_2O_2-induced decrease of the metabolic ATP level [14].

The active transport of serotonin (5-HT) across the platelet plasma membrane is also an energy-requiring function that has been the subject of several reviews [19–21]. Platelets accumulate 5-HT by two processes: active transport and passive diffusion. The contribution of the latter is negligible when the 5-HT concentration is below that required to saturate the transporter [20]. In addition to being energy-consuming, the active transport process has an absolute requirement for Na^+ and Cl^- in the medium [22–26]. Interest in the platelet 5-HT transport process is 2-fold. First, platelets have served as pharmacological and toxicological models for presynaptic serotonergic nerve terminals [21]. Second, the active transport of 5-HT by platelets is thought to be important in maintaining the circulating concentration of 5-HT below the levels required to activate vascular smooth muscle and platelet 5-HT receptors [27]. Alterations in platelet 5-HT accumulation have been observed in many vascular diseases in which 5-HT has been implicated [28, 29].

In light of the biochemical effects of H_2O_2 on human platelets and the importance of the 5-HT active uptake process, this study was undertaken to examine the effects of H_2O_2 on platelet 5-HT active transport.

* Abbreviations: H_2O_2, hydrogen peroxide; 5-HT, 5-hydroxytryptamine; ACD, citric acid–sodium citrate–glucose; PRP, platelet-rich plasma; G, β-D(+)glucose; GO, glucose oxidase; X, xanthine; XO, xanthine oxidase; and $O_2^{\cdot-}$, superoxide anion.

MATERIALS AND METHODS

Materials. Hydroxytryptamine binoxalate [1,2-^{3}H(N)] (29.7 Ci/mmol) was purchased from the New England Nuclear Corp. (Boston, MA), and its purity

was determined by thin-layer chromatography on 250-μm silica gel G plates in a solvent system composed of acetone/2-propanol/water/ammonium hydroxide (50:40:7:3). The radiochemical purity was greater than 97%. Liquid scintillation supplies were purchased from Research Products International (Downers Grove, IL). 5-Hydroxytryptamine oxalate, EDTA, β-D(+)glucose, xanthine, glucose oxidase (Type VII) from *Aspergillus niger*, xanthine oxidase (chromatographically purified from buttermilk), catalase from bovine liver, and superoxide dismutase from bovine liver were purchased from the Sigma Chemical Co. (St. Louis, MO). Fluoxetine hydrochloride was a gift from the Eli Lilly Co. (Indianapolis, IN). The buffer used in these experiments was composed of 0.1 M Na_2HPO_4 (pH 7.4) containing 5 mM KCl and was found to support 5-HT transport in a manner similar to Tyrode's buffer from which divalent cations had been excluded. This simple buffer contained the required ions for 5-HT transport [19, 20, 22–26] and provided excellent pH control.

Isolation and preparation of platelets. Blood (9 mL) was drawn from a normal, healthy volunteer into a plastic syringe containing 1 mL of 3% EDTA (w/v) as anticoagulant. Since the active accumulation of 5-HT is not dependent upon the extracellular calcium ion concentration, no alteration of uptake by the anticoagulants EDTA or ACD (citric acid–sodium citrate–glucose) would be expected, and this has been confirmed by us and others [30]. Platelet-rich plasma (PRP) was prepared by centrifugation of the samples at 200 *g* for 15 min at room temeprature. The PRP was removed, and the platelet concentration was determined using the Unopette System (Becton, Dickinson & Co., Rutherford, NJ) followed by counting with a Neubauer hemocytometer under a phase-contrast microscope. The PRP was diluted with buffer to give a final platelet concentration of 5×10^8/mL and was maintained at room temperature until used.

Exposure of platelets to H_2O_2. The PRP (100 μL, 5×10^7 platelets) was suspended in buffer (375 μL) and H_2O_2 (25 μL, 3.1 to 100 μM, final concentration) was added. Samples were then incubated for 0–16 min, rapidly cooled on ice, and centrifuged at 700 *g* for 10 min at 4°. The supernatant fraction was removed by careful aspiration, and the platelet pellet was resuspended in 200 μL of cold buffer and maintained in an ice bath until [^{3}H]5-HT uptake was measured. This was necessary because platelets maintained at room temperature recovered from the oxidant-induced stimulation. Control platelets were handled in an identical manner.

In experiments utilizing glucose oxidase, the PRP (100 μL, 5×10^7 platelets) was suspended in buffer (375 μL) containing 2.5 mM β-D(+)glucose. Glucose oxidase (25 μL, 0–100 milliunits) was added, and the samples were incubated for 0–16 min at 37° and the platelets isolated as described above.

In experiments using xanthine oxidase, the enzyme was first passed through a Sephadex G-50 fine centrifuge column as described by Penefsky [31]. This procedure removed both the 2.5 M $(NH_4)_4SO_4$ solution used to suspend xanthine oxidase and a low molecular weight (17,000 daltons) contaminant that caused platelet aggregation [32]. The PRP (100 μL, 5×10^7 platelets) was added to buffer (375 μL) containing 0.5 mM xanthine. Xanthine oxidase (25 μL, 50 milliunits) was added, and the samples were incubated for 2 min at 37°. Platelets were isolated as described above. In certain experiments, catalase (1000 units/mL), superoxide dismutase (250 units/mL), heat-denatured (95°/10 min) glucose oxidase or xanthine was present in the initial buffer.

Platelet [^{3}H]5-*HT uptake.* After a 2-min preincubation, [^{3}H]5-HT (50 μL, 5×10^{-7} M final concentration, 100 nCi) was added, and the incubation was continued for 4 min at 37° in air. In certain experiments, fluoxetine (1 μM) was present in the buffer at the time of preincubation. The final 5-HT concentration in the kinetic experiments ranged from 2×10^{-6} M to 10^{-8} M. Human platelets accumulated 5-HT in a linear manner for 4 min.

The uptake was terminated by the addition of 1.0 mL of a cold solution of 0.1% EDTA (w/v) in isotonic saline containing 1 μM fluoxetine hydrochloride, followed by rapid cooling on ice. The platelets were pelleted from solution by centrifugation of the sample at 3500 *g* for 10 min at 4°. The supernatant fraction was removed, and the platelet pellet was solubilized in 200 μL of 15% H_2O_2 by vortexing and incubating the sample for 15 min at 37°. A 100-μL aliquot of the sample was added to scintillation vials containing 5 mL of scintillation fluid and counted.

The kinetic data were analyzed by the method of Stahl and Meltzer [33], utilizing an Eadie–Hofstee plot for the determination of K_m and V_{max} values.

Measurement of H_2O_2 *production by glucose oxidase.* This spectrophotometric procedure was provided by the enzyme supplier and is described below briefly. In a cuvette, 2.40 mL of *o*-dianisidine (2.1 mM) in buffer (pH 7.4), 0.50 mL β-D(+)glucose (10%, w/v) and 0.10 mL peroxidase solution (60 purpurogallin units/mL) were mixed and equilibrated at 37°. The A_{500} of the sample versus air was monitored until a steady baseline was achieved and 0.10 mL of glucose oxidase solution (1.25 to 100 milliunits) added. The increase in A_{500} versus air was recorded for 2–4 min, and a plot of A_{500} versus time was used to determine the maximum linear rate of H_2O_2 production.

Statistical methods. Platelet [^{3}H]5-HT uptake data are expressed as means ± SE. Statistical differences were determined using either a one-way analysis of variance followed by Dunnett's test of an unpaired Student's *t*-test with $P < 0.05$ as the limit for significant differences [34]. The platelet kinetic data have been expressed as means ± SD, and the determination of significance was based on the calculation of the 95% confidence band around each plot following linear regression analysis of the data [35].

RESULTS

Effect of H_2O_2 *on human platelet 5-HT transport.* The treatment of platelets with increasing concentrations of H_2O_2 (0–100 μM) produced a marked stimulation of 5-HT accumulation and a clear concentration–response relationship (Table 1) which was significant when subjected to linear regression

Table 1. Concentration–response of H_2O_2 stimulation of human platelet 5-HT uptake

Concentration of H_2O_2 (μM)	Uptake (pmol/10^8 platelets/4 min)
0	40.6 ± 0.5
3.12	39.0 ± 0.2
6.25	42.4 ± 0.3
12.5	52.2 ± 1.0*
25	52.4 ± 2.4*
50	54.4 ± 0.5*
50 + Catalase (1000 units)	42.3 ± 0.8
50 + Fluoxetine (1 μM)	5.9 ± 0.1*
100	53.0 ± 1.4*

Human platelets (5×10^7) in 0.375 mL of buffer (0.5 mL final volume) were incubated for 2 min at 37° after the addition of H_2O_2. Platelets were isolated, and [^{3}H]5-HT uptake was measured as described in Materials and Methods. Values are means ± SE, N = 3. Control 5-HT uptake values were not altered significantly in the presence of catalase (1000 units).

* Significantly different from control (0 μM H_2O_2), $P < 0.05$.

Table 2. Time-course of H_2O_2 stimulation of human platelet 5-HT uptake

Time (min)	Treatment	Uptake (pmol/10^8 platelets/4 min)
0	Control	49.8 ± 0.9
	H_2O_2	50.4 ± 0.5
1	Control	53.8 ± 0.6
	H_2O_2	57.3 ± 0.4*
2	Control	50.2 ± 0.4
	H_2O_2	70.0 ± 1.2*
3	Control	46.7 ± 0.4
	H_2O_2	65.3 ± 1.1*
4	Control	42.1 ± 0.5
	H_2O_2	66.8 ± 1.4*
8	Control	31.9 ± 0.7
	H_2O_2	54.4 ± 0.8*
16	Control	28.6 ± 0.1
	H_2O_2	45.3 ± 0.6*

Human platelets (5×10^7) in 0.375 mL of buffer (0.5 mL final volume) were incubated for the specified times at 37° after the addition of H_2O or H_2O_2 (50 μM final concentration). Platelets were isolated, and [^{3}H]5-HT uptake was measured as described in Materials and Methods. Values are means ± SE, N = 3.

* Significantly different from control, $P < 0.05$.

analysis ($r = 0.93$, $P < 0.05$). A H_2O_2 concentration as low as 12.5 μM significantly stimulated 5-HT accumulation. The addition of catalase, which converts H_2O_2 to H_2O and O_2, to the incubation medium completely prevented the H_2O_2-induced stimulation of this transport process (Table 1). The addition of fluoxetine, a specific inhibitor of the active transport component of platelet 5-HT accumulation [36], before 5-HT, completely prevented the active transport of 5-HT into H_2O_2-stimulated platelets (Table 1). No change in the passive diffusion component of 5-HT accumulation was noted between control and H_2O_2-treated human platelets (data not shown).

The stimulation of platelet 5-HT active transport was time dependent, with maximal stimulation being observed following a 2-min incubation (Table 2). When platelets were preincubated (0–10 min) or incubated (2–16 min), there was a decrease in the observed extent of basal 5-HT transport. No preincubation of samples and a 2-min incubation following the addition of H_2O_2 was found to be optimal and was used throughout these studies. These conditions were specifically chosen to avoid incubation effects.

Effect of β-D(+)glucose/glucose oxidase generated H_2O_2 on human platelet 5-HT transport. The glucose/glucose oxidase system has been used frequently to generate H_2O_2 continuously. When glucose in pH 7.4 buffer was treated with increasing amounts of glucose oxidase (0–100 milliunits), a clear concentration–response relationship was observed (Table 3) which was significant when subjected to linear regression analysis ($r = 0.92$, $P < 0.05$). Exposure of human platelets to this system produced a similar concentration–response to that observed with the bolus addition of H_2O_2 (Table 3). One difference between the two systems was that low concentrations of glucose/glucose oxidase generated

Table 3. Concentration–response of glucose/glucose oxidase generated H_2O_2 on the stimulation of human platelet 5-HT uptake

Glucose oxidase (milliunits)	H_2O_2 generation (nmol/min)	Uptake (pmol/10^8 platelets/4 min)
0		43.3 ± 1.2
3.12	0.5	36.1 ± 0.9*
6.25	1.0	38.2 ± 0.6
12.5	2.1	40.5 ± 0.4
25	4.1	49.3 ± 0.3*
50	8.4	56.0 ± 2.6*
100	17.2	58.6 ± 0.6*
100 + Catalase (1000 units)		43.0 ± 0.5
100 + Fluoxetine (1 μM)		4.3 ± 0.1*

Human platelets (5×10^7) in 0.375 mL of buffer (0.5 mL final volume) containing 2.5 mM β-D(+)glucose were incubated for 2 min at 37° after the addition of different amounts of glucose oxidase. Platelets were isolated, and [^{3}H]5-HT uptake was measured as described in Materials and Methods. Values are means ± SE, N = 3. Control 5-HT uptake values were not altered significantly in the presence of catalase (1000 units).

* Significantly different from control (0 milliunits glucose oxidase), $P < 0.05$.

Table 4. Time-course of β-D(+) glucose (G)/glucose oxidase (GO) generated H_2O_2 on the stimulation of human platelet 5-HT uptake

Time (min)	Treatment	Uptake (pmol/10^8 platelets/4 min)
0	Control	36.8 ± 0.5
	G/GO	40.5 ± 0.3*
1	Control	38.5 ± 0.7
	G/GO	49.9 ± 0.9*
2	Control	36.4 ± 0.6
	G/GO	51.2 ± 0.9*
2	−G/+GO	35.3 ± 0.3
2	+G/Heat-denature enzyme	34.3 ± 0.7
4	Control	29.6 ± 0.5
	G/GO	40.8 ± 0.0*
8	Control	24.0 ± 0.2
	G/GO	37.5 ± 0.7*
16	Control	21.3 ± 0.0
	G/GO	36.7 ± 0.1*

Human platelets (5×10^7) in 0.375 mL of buffer (0.5 mL final volume) containing 5.0 mM β-D(+) glucose were incubated for various times at 37° after the addition of 50 milliunits of glucose oxidase. Platelets were isolated, and [^{3}H]5-HT uptake was measured as described in Materials and Methods. Values are means ± SE, N = 3.
* Significantly different from control, $P < 0.05$.

H_2O_2 had an inhibitory effect on platelet 5-HT uptake (Table 3) which was not observed with low concentrations of H_2O_2 added as a bolus (Table 1). The glucose/glucose oxidase system produced H_2O_2 in the human platelet samples in a concentration-dependent manner (Table 3). Assuming no degradation, glucose oxidase (100 milliunits) would produce a final H_2O_2 concentration of 68.8 μM in these samples after a 2-min incubation. The addition of catalase to the incubation medium prevented the stimulation which indicated that H_2O_2 was responsible for the stimulation and not a component of the generating system (Table 3). This latter point was further confirmed by showing that platelets exposed to glucose oxidase in the absence of glucose, heat-denatured glucose oxidase in the presence of glucose (Table 4), or glucose in the absence of glucose oxidase (data not shown) failed to be stimulated. Fluoxetine was also able to block completely the active transport of glucose/glucose oxidase treated platelets which further demonstrated that H_2O_2 was affecting only the active transport component of 5-HT accumulation (Table 3).

The exposure of human platelets to H_2O_2 generated by glucose/glucose oxidase showed a similar time-course of stimulation to that observed following a bolus addition of H_2O_2 (Table 4). Maximal stimulation of 5-HT transport was found following a 2-min incubation. With continued incubation (2–16 min) there was a decrease in 5-HT transport by both control and H_2O_2-exposed platelets.

Effect on human platelet 5-HT transport of xanthine/xanthine oxidase generated $O_2^{\cdot-}$ and H_2O_2. This enzyme system has been used extensively to generate $O_2^{\cdot-}$ which can spontaneously or enzymatically dismutate to H_2O_2 [1]. When human platelets were exposed to this system, a prompt, marked stimulation of platelet 5-HT accumulation was observed (Table 5). Previous work from this laboratory has shown that under these conditions xanthine oxidase (50 milliunits) generated 85.2 nmol $O_2^{\cdot-}$/min [37]. Assuming that all the $O_2^{\cdot-}$ produced in a 2-min incubation was dismutated to H_2O_2 and was not degraded, a final maximal H_2O_2 concentration of 85.2 μM would be achieved in the sample. The stimulation required all components of the generating system and was prevented when substrate, enzyme or heat-denatured enzyme was used (Table 5). When superoxide dismutase, which catalyzed the dismutation of $O_2^{\cdot-}$ to H_2O_2, was added to the platelet suspension, full stimulation of 5-HT transport was observed. This suggested that H_2O_2 was the oxidant responsible for the stimulation observed with this system, and its effects could be prevented by catalase (Table 5). When fluoxetine was added to the uptake medium of platelets stimulated with the xanthine/xanthine oxidase system, 5-HT uptake was blocked. This indicated that the effect of H_2O_2 was on the platelet active transport mechanism and was not the result of an altered plasma membrane.

Effect of H_2O_2 *on human platelet 5-HT transport kinetics.* The kinetics of human platelet 5-HT transport were studied in order to further gain information on the mechanism of H_2O_2-induced stimulation (Fig.

Table 5. Effect of xanthine (X)/xanthine oxidase (XO) generated $O_2^{\cdot-}$ and H_2O_2 on the stimulation of human platelet 5-HT uptake

Treatment	Uptake (pmol/10^8 platelets/4 min)
X/−XO	31.8 ± 0.2
−X/XO	32.8 ± 0.7
X/XO	44.0 ± 0.4*
X/XO + superoxide dismutase (250 units)	42.0 ± 0.4*
X/XO + catalase (1000 units)	31.5 ± 0.3
X/Heat-denatured XO	29.8 ± 0.4*
X/XO + fluoxetine (1 μM)	3.7 ± 0.1*

Human platelets (5×10^7) in 0.375 mL of buffer (0.5 mL final volume) containing 0.5 mM xanthine were incubated for 2 min at 37° after the addition of 50 milliunits of xanthine oxidase. Platelets were isolated, and [^{3}H]5-HT uptake was measured as described in Materials and Methods. Values are means ± SE, N = 3. Control 5-HT uptake values were not altered significantly in the presence of superoxide dismutase (250 units) or catalase (1000 units).
* Significantly different from control (X/−XO), $P < 0.05$.

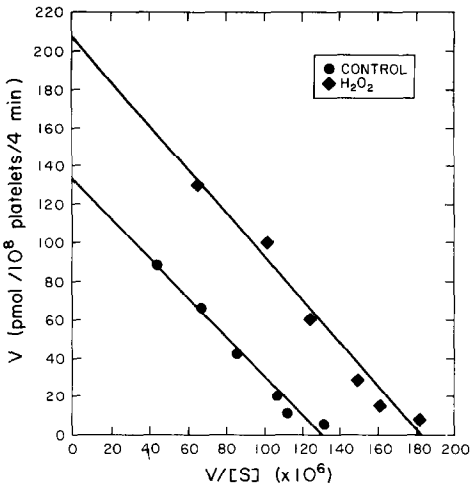

Fig. 1. Eadie–Hofstee plot of saturable, active component of 5-HT uptake obtained with control and H_2O_2-treated human platelets. Linear regression analysis of the data gave intercepts (V_{max}) control = 131.4 and H_2O_2 = 206.7; slopes ($-K_m$) control = 1.0 and H_2O_2 = 1.1; and correlations (r) control = 0.987 and H_2O_2 = 0.986. The intercepts (V_{max}) were significantly different.

1). No significant difference was observed in the K_m values (K_m control = $1.0 \pm 0.2 \times 10^{-6}$ M vs K_m H_2O_2 = $1.1 \pm 0.1 \times 10^{-6}$ M) but a marked increase was found in the V_{max} value of H_2O_2-treated platelets when compared to control platelets (V_{max} control = 131.4 ± 4.6 pmol/10^8 platelets/4 min vs V_{max} H_2O_2 = 206.7 ± 9.1 pmol/10^8 platelets/4 min), $P < 0.01$. Thus, H_2O_2-stimulated human platelets have a significant increase in their maximal rate of 5-HT transport.

DISCUSSION

The results of this study are the first to have demonstrated that the 5-HT transport system in human platelets is stimulated significantly by exposure to potentially physiological concentrations of H_2O_2. This effect could be achieved by either the direct addition of H_2O_2 to the platelet suspension or by the continuous generation of H_2O_2 by the enzyme reaction of glucose with glucose oxidase. This latter procedure resulted in a continuous generation of H_2O_2 throughout the incubation period and was used because human platelet function has been reported to be more sensitive to continuously generated H_2O_2 [18]. In addition, the xanthine/xanthine oxidase system was used to continuously produce $O_2^{\cdot-}$ which can undergo enzymatic or non-enzymatic dismutation to H_2O_2 and O_2. When these procedures were used to expose human platelets to H_2O_2, a similar effect was observed: a marked, rapid stimulation of 5-HT transport that was maximal after 2 min of incubation (Tables 2, 4 and 5). With continued incubation, there was a reduction in 5-HT uptake which has been described previously and is known as the "pre-incubation effect" [19]. The rapid increase in platelet 5-HT uptake that occurs in response to an oxidant such as H_2O_2 raises the possibility that this process may represent a regulatory mechanism which platelets utilize to reduce the extracellular concentration of 5-HT when exposed to oxidants at sites of inflammation and injury where aggregation and 5-HT release have occurred.

A concentration–response relationship was evident between H_2O_2 concentration and the stimulation of transport (Tables 1 and 3). The effective H_2O_2 concentration was considerably lower than those reported to cause changes in platelet ultrastructure and function [13, 14, 17]. Indeed, exposure of platelets to 12.5 μM H_2O_2 was sufficient to cause a significant stimulation of 5-HT transport (Table 1). Human platelets contain the intracellular antioxidant enzymes superoxide dismutase, catalase and glutathione peroxidase which protect sensitive intracellular organelles from oxidant injury [8, 9]. The high concentrations of H_2O_2 needed to affect platelet ultrastructure and function may be due to the necessity to overwhelm these antioxidant defense enzymes. In contrast, the lower H_2O_2 concentrations required to stimulate the platelet 5-HT transport system suggest that H_2O_2 may be affecting the platelet plasma membrane or sites not protected by catalase and glutathione. In fact, it has been observed that in spite of active H_2O_2 degradation, platelets may not be able to protect some sensitive intracellular sites upon exposure to H_2O_2 [16].

The oxidant species responsible for the stimulation of the human platelet 5-HT transport system appears to be H_2O_2; however, the involvement of the HO$^{\cdot}$ radical cannot be ruled out completely. This radical can be formed via a Fenton reaction involving H_2O_2 and a transition metal [1, 3, 38]. Alternatively, HO$^{\cdot}$ can be formed by a metal-catalyzed Haber–Weiss reaction which involves both $O_2^{\cdot-}$ and H_2O_2 [38]. When xanthine/xanthine oxidase was used to generate $O_2^{\cdot-}$ and H_2O_2, superoxide dismutase had no effect on the stimulation of the active transport of 5-HT by platelets (Table 5). Since superoxide dismutase catalyzes the dismutation of $O_2^{\cdot-}$ to H_2O_2 and O_2, this would suggest that H_2O_2 was the oxidant species responsible for the observed stimulation and not $O_2^{\cdot-}$.

The kinetics of human platelet 5-HT transport were measured in order to further understand the mechanism of H_2O_2-induced stimulation of this process. The kinetics were analyzed using the method of Stahl and Meltzer [33] and indicated that H_2O_2 significantly increased the V_{max} without affecting the K_m of transport (Fig. 1). Various diseases such as hypertension [39, 40], adult respiratory distress syndrome [41], and depression [30, 42] have been associated with altered platelet 5-HT uptake kinetics in which decreased K_m and/or V_{max} values have been observed. An increase in V_{max} has not been reported.

Based on these data, it is possible to suggest several mechanisms by which the stimulation of 5-HT transport may occur. One mechanism could be through H_2O_2 causing a direct or indirect effect on Na^+-K^+-ATPase. The active transport of 5-HT by human platelets appears to be linked to Na^+-K^+-ATPase [43], and a direct stimulation of this membrane bound, protein thiol enzyme by H_2O_2 could result

in an increase in 5-HT transport. Such a direct stimulation of Na^+-K^+-ATPase might be expected to decrease the metabolic pool of ATP, and this has been observed following the addition of H_2O_2 to human platelets [14]. Alternatively, H_2O_2 may stimulate this enzyme indirectly through its ability to block the effects of plasma Na^+-K^+-ATPase inhibitors. Several such inhibitors have been described and have been shown to inhibit platelet 5-HT in a ouabain-like manner [44–66].

A second mechanism by which H_2O_2 may cause the marked increase in platelet 5-HT transport could be through an increase in the number of functional transporter complexes. This could occur through the reorientation or migration of inactive transporter molecules to the outer platelet membrane, thereby increasing the number of available transporter molecules and increasing the V_{max} of uptake. A similar process has been described for the stimulation of glucose transport by insulin [47].

Lastly, exposure of platelets to H_2O_2 may cause an increase in plasma membrane fluidity either as a result of lipid peroxidation or by decreasing the sulfhydryl content of the membrane. Changes in plasma membrane fluidity have been noted following exposure of cultured endothelial cells to the xanthine/xanthine oxidase generating system [48]. Such an increase in membrane fluidity could result in the more facile movement of the transporter complex through the membrane, thereby increasing the transporter turnover number. A change in membrane fluidity would be consistent with these data that found H_2O_2 to affect only the active component of 5-HT accumulation without altering the passive diffusion component (Tables 1 and 3).

In summary, these data have shown that human platelets stimulate the active transporter of 5-HT in response to exposure to micromolar concentrations of H_2O_2. It is possible that H_2O_2 may function to regulate the active transport of 5-HT by human platelets since platelets possess a membrane bound, NADH-dependent H_2O_2 generating system which is activated by various stimuli [49]. The mechanism and significance of this process are currently under investigation.

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