The Effect of Quinidine on the Uptake and Release of Serotonin by Platelets *in Vitro** (34048)

JAMES W. DAVIS[1] AND SLOAN J. WILSON

Section of Hematology, Department of Medicine, University of Kansas School of Medicine, Kansas City, Kansas 66103 and the Veterans Administration Hospital, Kansas City, Missouri 64128

Both human (1) and guinea pig (2) platelets are capable of taking up serotonin *in vitro* against concentration gradients of approximately 1000 to 1. Serotonin uptake is temperature dependent and appears to be an active process (1–3). This report shows that like reserpine (2–4) quinidine noncompetitively inhibits the uptake of serotonin by platelets and causes its release from platelets.

Materials and Methods. Platelet-rich plasma (PRP) was obtained by the centrifugation at 500*g* for 20 min at 4° of 29 parts of normal human blood mixed with 1 part of 5% disodium ethylenediaminetetraacetate (EDTA) in 0.9% sodium chloride solution (saline) in siliconized glass tubes with the exception that in one set of experiments a final concentration of 0.38% trisodium citrate was the anticoagulant.

5-Hydroxy-3-indolyl (ethyl-2-amine-1-^{14}C) creatinine sulfate monohydrate (serotonin-^{14}C) with a specific activity of 32 mC/mmole was obtained from Nuclear-Chicago. Quinidine sulfate was dissolved in saline solutions of serotonin-^{14}C. One milliliter aliquots of the saline solutions were mixed with 1-ml aliquots of PRP and incubated at 37° for 1 hr. The final concentration of serotonin-^{14}C in the reaction mixtures was 1.56 $\times$ 10^{-6} *M*. Platelets were separated by centrifugation at 2000*g* for 30 min at 4°. The liquid was decanted, and the platelet plugs were washed with 3 ml of saline and centrifuged again. The tubes were inverted for 2 hr before residual liquid was wiped from the walls of the tubes. Platelet plugs were then dissolved by mixing them with 1 ml of 1.0 *M* *p*-(diisobutyl-cresoxyethoxyethyl) dimethylbenzylammonium hydroxide (Hydroxide of Hyamine 10-X, Packard) and incubating the mixture at 56° for 20 hr. Platelet solutions were washed from the tubes with 18 ml of a scintillation solution prepared by dissolving 5.0 g of 2,5-diphenyloxazole (PPO, Packard) and 0.3 g of 1, 4-bis-[2-(5-phenyloxazolyl)]-benzene (POPOP, Packard) in 1000 ml of toluene. Radioactivity of the platelet solutions was measured using a Packard Tri-Carb liquid scintillation spectrometer. The radioactivity of solutions prepared from platelets which had been incubated with quinidine was divided by the radioactivity of a solution prepared from platelets which had been incubated without quinidine. Each of the resulting percentages was subtracted from 100% to give the percentage of inhibition of serotonin-^{14}C uptake.

In order to prepare a Lineweaver-Burk plot we used four pairs of solutions containing different concentrations of serotonin-^{14}C. One of each pair of solutions also contained quinidine sulfate. One milliliter of PRP, preincubated at 37°, was mixed with 1 ml of each of the eight solutions which were also preincubated at 37°. Final concentrations of serotonin-^{14}C were 0.39, 0.78, 1.56, and 3.12 $\times$ 10^{-6} *M* respectively. Four of the mixtures contained quinidine sulfate at a final concen-

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[1] Clinical Investigator, Veterans Administration Hospital, 4801 Linwood Blvd., Kansas City, Missouri 64128 (address reprint requests to Dr. Davis).

tration of 4×10^{-6} *M* (3.125 mg/liter). After 10 min at 37° the tubes containing the eight mixtures were placed in ice water. Platelet solutions were prepared and their radioactivity measured as described above.

The release of serotonin-^{14}C from platelets was determined after 1-ml portions of PRP were preincubated at 37° for 2 hr with 1 ml of serotonin-^{14}C solution (3.12×10^{-6} *M*). One milliliter of saline with or without quinidine sulfate was then added. After incubation with saline at 37, 22, and 0° for 4 hr platelet radioactivity declined by only 2, 4, and 5% respectively. The radioactivity of solutions prepared from platelets which had been incubated with quinidine for 4 hr was divided by the radioactivity of a solution prepared from platelets which had been incubated with saline at the corresponding temperature. Each of the resulting percentages was subtracted from 100% to give the percentages of release of serotonin-^{14}C by quinidine.

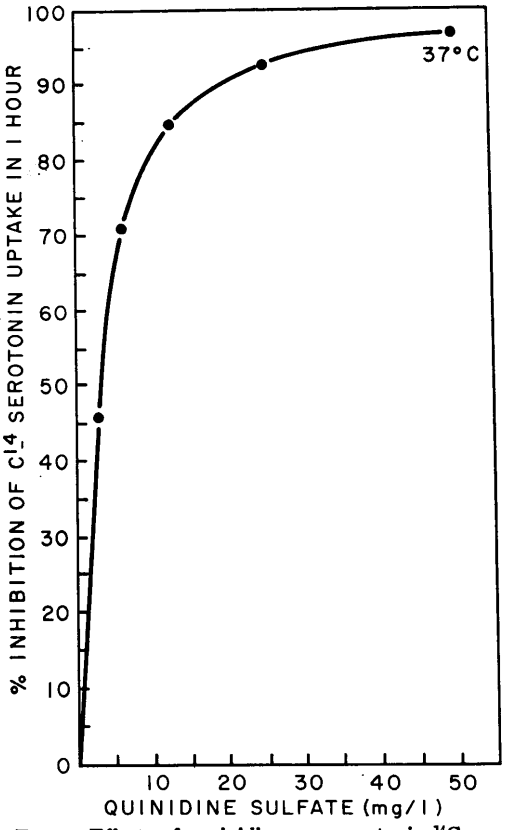

FIG. 1. Effect of quinidine on serotonin-^{14}C uptake by platelets in EDTA PRP.

Results. The uptake of serotonin-^{14}C by the platelets of normal human PRP was inhibited by quinidine sulfate (Fig. 1). A therapeutic concentration of 6.25 mg/liter caused 71% inhibition. In another set of experiments citrate was used as the anticoagulant, and there was no inhibition of serotonin uptake by 6.25 mg/liter of quinidine sulfate and 41 and 68% inhibition by 25 and 50 mg/liter respectively.

There was essentially no change in the percentage of inhibition (approximately 80%) of serotonin-^{14}C uptake of platelets by a constant concentration of quinidine sulfate when the serotonin-^{14}C concentration was repeatedly doubled. The lack of decreased inhibition as serotonin concentration was increased suggests that quinidine inhibits serotonin uptake noncompetitively. This was confirmed when the data were plotted by the method of Lineweaver and Burk (5). The regression lines representing solutions with and without quinidine intercepted the ordinate at different points (Fig. 2).

Quinidine sulfate released serotonin from the platelets of normal human PRP at 37° and to a lesser extent at 22°, but not at 0° (Fig. 3).

To determine whether quinidine causes lysis of platelets, PRP was mixed with quinidine sulfate solution in saline to achieve a final concentration of 75 mg of quinidine sulfate per liter. The mixture was incubated at 37° for 2 hr and then at 26° for another 4 hr. There was no decrease in platelet count done by phase-contrast microscopy after 2 hr and 6 hr of incubation, and no platelet clumps were seen.

Discussion. Guinea pig (2) and human (3) platelets take up serotonin by active transport and by simple diffusion. Diffusion predominates at high concentrations of serotonin, but at low concentrations, such as we used, uptake is largely by active transport.

Several compounds compete with serotonin for uptake by platelets. Lineweaver-Burk plots characteristic of competitive inhibition have been published for chlorpromazine, cocaine, desmethylimipramine, imipramine,

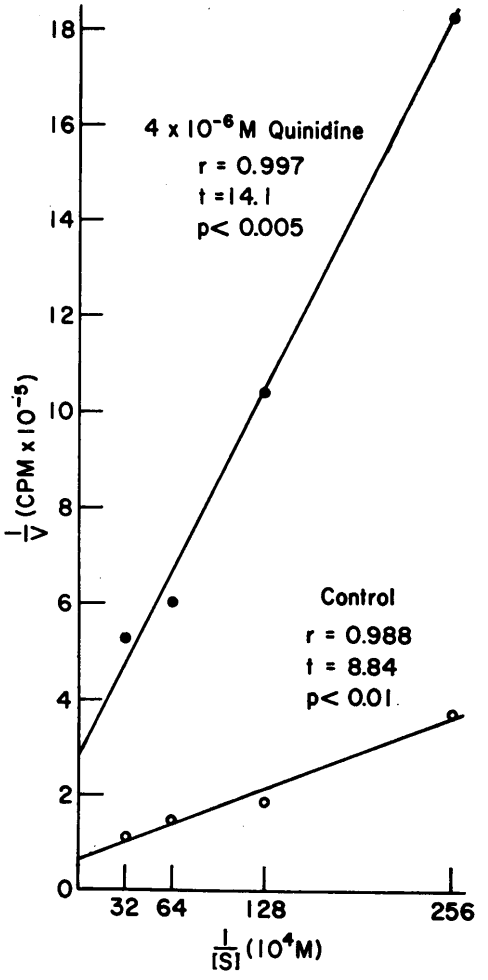

FIG. 2. Abscissa: The reciprocal of the concentration of serotonin-^{14}C added to PRP. Ordinate: The reciprocal of the velocity of uptake (cpm) of serotonin-^{14}C during a 10-min incubation. Correlation coefficients are shown for each regression line.

and tryptamine (3, 6). To our knowledge reserpine is the only compound previously reported to inhibit serotonin uptake noncompetitively (3). In Fig. 2 the regression lines representing solutions with and without quinidine do not intercept at the same maximum velocity. This is characteristic of noncompetitive inhibition. We found that serotonin uptake by human platelets was progressively inhibited (Fig. 1) from 46 to 97% as quinidine sulfate concentration was increased from 3 to 50 mg per liter (4×10^{-6} to 6.4×10^{-5} *M*). In contrast Fuks *et al.* (3) using methods similar to ours found that although reserpine was a very potent inhibitor of serotonin uptake by human platelets, with 87% inhibition achieved at a reserpine concentration of 2×10^{-6} *M*, no further inhibition occurred at higher reserpine concentrations.

Like reserpine (4, 7), quinidine releases little serotonin from platelets at low temperatures (Fig. 3). Hughes and Brodie (2) suggested that when the active uptake of serotonin is blocked, this poorly lipid-soluble substance is slowly released by passive diffusion through the platelet membrane. This explanation of serotonin release at first seemed incompatible with our observation that exposure of platelets to 0° for 4 hr caused negligible serotonin release and with the findings that both reserpine-induced (4, 7) and quinidine-induced (Fig. 3) release of serotonin from platelets are inhibited by lowering the temperature. These observations seemed more compatible with the suggestion of Buckingham and Maynert (7) that sero-

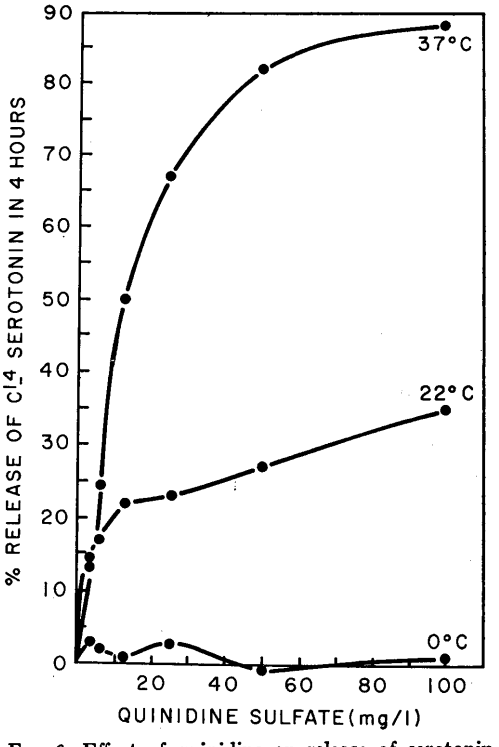

FIG. 3. Effect of quinidine on release of serotonin-^{14}C from platelets in EDTA PRP.

tonin release may be an active process inhibited by cold and set into motion by releasing agents. However, Pletscher (8) has recently reported that serotonin release by such drugs as reserpine is not inhibited by metabolic inhibitors as is the active uptake of serotonin by platelets. Pletscher *et al.* (9) found that the outflow of serotonin from platelets in the presence of metabolic inhibitors or in a glucose-free potassium phosphate buffer (where active transport was not thought to be possible) was diminished at low temperatures. It was suspected that lowering the temperature might interfere with passive diffusion of serotonin through platelet membranes by altering the physicochemical properties of certain membrane constituents.

Quinidine appears to release serotonin from platelets more rapidly than reserpine. We found a progressively increasing release of serotonin as quinidine concentration was increased with 88% release occurring in 4 hr with 1.3×10^{-4} *M* quinidine (Fig. 3). Carlsson *et al.* (4) reported that a maximally effective concentration of reserpine (4.8×10^{-7} *M*) released only about 50% of the serotonin content of rabbit platelets in 4 hr. Pletscher and Tranzer (10) found that 1.6×10^{-6} *M* reserpine released 53% and 78% of the serotonin content of cat platelets after 6 and 12 hr respectively. The slower releasing effect of reserpine may be due to an interference with the outflux of serotonin through the external membranes of platelets (11) after its release from subcellular storage particles (10).

It appears likely that EDTA, which may cause as much as 30% increase in platelet volume (12), also potentiates the inhibition of serotonin uptake by low concentrations of quinidine. Although quinidine sulfate in concentrations of 3.125 and 6.25 mg/liter (readily achieved in humans) caused 46 and 71% inhibition respectively of serotonin uptake by the platelets of EDTA PRP, approximately eight times these concentrations were required to achieve equivalent inhibition in citrated PRP. This makes it seem unlikely that therapeutic concentrations of quinidine would inhibit serotonin uptake by platelets *in vivo*.

Bridges *et al.* (13) found the inhibition by serum of platelet uptake of serotonin to be a sensitive technique for the detection of platelet isoantibodies. It is apparent from the foregoing discussion that such a technique would not be suitable for detection of antibodies against quinidine or other drugs which are potent inhibitors of serotonin uptake.

Summary. The addition of quinidine sulfate to human platelet-rich plasma inhibited serotonin uptake by platelets and caused the release of serotonin from platelets. Kinetic data suggest that the inhibition of serotonin uptake is noncompetitive. The release of serotonin by quinidine can be prevented by lowering the temperature.

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