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Rapid communication

Tyrosine kinase inhibitors regulate serotonin uptake in platelets

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Abstract

Uptake of tritiated serotonin into human platelets was found to be rapidly inhibited by the tyrosine kinase inhibitors, genistein and methyl 2,5-dihydroxycinnamate. Binding studies indicated that uptake inhibition did not correlate with direct binding of these inhibitors to the transporter. Chelation of mobilizable intracellular Ca^{2+} did not inhibit the effects of genistein on uptake. These results suggest a more direct, non-Ca^{2+} mediated effect of tyrosine kinase inhibitors on uptake.

Keywords: Genistein; Erbstatin analog; Ca^{2+}

Regulation of serotonin transport has been a major focus of antidepressant research, with many specific serotonin uptake inhibitors being effective antidepressants. In view of data suggesting abnormal platelet function in the affective disorders (with regard to serotonin uptake and intracellular Ca^{2+}), elucidation of pathways involved in regulation of serotonin uptake is needed to understand the underlying mechanisms involved in these abnormalities. Early reports have demonstrated unusually high levels of tyrosine kinases in platelets (Golden et al., 1986). We now report that serotonin uptake in human platelets is rapidly decreased by tyrosine kinase inhibitors, suggesting that platelet tyrosine kinase activity has tight control over serotonin uptake.

Two broad spectrum tyrosine kinase inhibitors, genistein (4[1],5,7-trihydroxyisoflavone) and erbstatin analog (methyl 2,5-dihydroxycinnamate), were used. Genistein (Res. Biochem. Internat., Natick, MA, USA) is a competitive inhibitor of adenosine triphosphate (ATP) in the kinase reaction and is noncompetitive with regard to substrate. Methyl 2,5-dihydroxycinnamate (Res. Biochem. Internat., Natick, MA, USA) is an analog of erbstatin which is competitive with both substrate and ATP (Levitzki and Gazit, 1995).

Uptake of 100 nM [^{3}H]5-hydroxytryptamine (serotonin) (Amersham, Arlington Heights, IL, USA, 57 Ci/mmol) in human platelets (supplied by the Los Angeles Red Cross) was performed in Krebs-Ringer Hepes buffer (145 mM NaCl, 5 mM KCl, 0.5 mM Na_2HPO_4, 1 mM $MgSO_4$, 0.2 mM $CaCl_2$, 10 mM Hepes (*N*-[2-hydroxyethyl]piperazine-*N*'[2-ethanesulfonic acid]), 10 mM glucose, pH 7.4), using 100 μM desmethylimipramine (Res. Biochem. Internat., Natick, MA, USA) to define specific uptake (5 min, 37°C). Platelets (5×10^7/ml to 10^8/ml) were incubated (37°C) with varying concentrations of kinase inhibitors for times varying from 1 to 30 min. [*N*-Methyl-^{3}H]Imipramine (0.1 nM, New England Nuclear, Boston, MA, USA, 85.3 Ci/mmol) binding to platelet membranes was performed in Krebs-Ringer Hepes buffer (4°C) and incubated on ice (4°C) for 2 h before separation of bound from free ligand by vacuum filtration through GF/B glass fiber filters (Whatman, UK). Protein was determined by the Bradford method (Bradford, 1976). Common chemicals were from Sigma Chem. Co. (St. Louis, MO, USA) and Calbiochem (La Jolla, CA, USA).

As shown in Table 1, both genistein and methyl 2,5-dihydroxycinnamate cause time-and dose-dependent decreases in serotonin uptake. The effects of methyl 2,5-dihydroxycinnamate, in contrast with the effects of genistein, were more transient, in keeping with the short half-life of methyl 2,5-dihydroxycinnamate in serum (i.e., approximately 50% of this com-

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Table 1
[^{3}H]Serotonin uptake (% control, mean ± S.E.M., $n = 3$–10)

Compound	1 min	5 min	10 min	15 min	30 min
(A) Time course					
Genistein (100 μM)	68 ± 5% [a]	13 ± 4 [d]	15 ± 0.5 [d]	9 ± 4 [c]	14 ± 3 [c]
Methyl 2,5-dihydroxycinnamate (100 μM)	98 ± 7	54 ± 6 [b]	74 ± 6 [a]	70 ± 4 [b]	95 ± 1
	0.1 μM	1 μM	10 μM	100 μM	
(B) Dose response (37°C, 10 min)					
Genistein	97 ± 2%	80 ± 5	75 ± 2 [d]	19 ± 6 [c]	
Methyl 2,5-dihydroxycinnamate	97 ± 7	89 ± 4	93 ± 4	74 ± 6 [a]	
	0.1 μM	1 μM	10 μM	100 μM	
(C) Inhibition of specific [^{3}H]imipramine binding (2 h, 4°C incubation)					
Genistein	97 ± 4%	107 ± 7	99 ± 3	104 ± 2	
Methyl 2,5-dihydroxycinnamate	96 ± 2	100 ± 3	95 ± 4	71 ± 3 [d]	

[a] $P < 0.05$ (paired T-test), [b] $P < 0.025$, [c] $P < 0.005$, [d] $P < 0.001$.

pound degrades within the first 30 min of incubation at 37°C) (Umezawa et al., 1990).

Maximum inhibition of uptake occurred by 5 min for both compounds. This inhibition did not correlate with inhibition of [^{3}H]imipramine binding to the serotonin transporter (Table 1, part C), suggesting that uptake inhibition was not due to direct binding of genistein or methyl 2,5-dihydroxycinnamate to the transporter.

Tyrosine kinase inhibition is known to affect Ca^{2+} fluxes in platelets, which in themselves may modulate serotonin uptake (Nishio et al., 1995). To determine whether changes in mobilizable Ca^{2+} mediate genistein's inhibition of serotonin uptake, platelets were pre-incubated with BAPTA-AM (acetoxymethyl bis (*O*-aminophenoxy) ethane-*N*,*N*,*N*,*N*-tetraacetate) (Calbiochem, La Jolla, CA, USA) (10 μM, 37°C, 30 min), a chelator of intracellular Ca^{2+}. This concentration of BAPTA-AM was sufficient to prevent detection of thrombin-mediated intracellular Ca^{2+} elevations in platelets (as measured by the fura-2 method, data not shown), suggesting that mobilizable intracellular Ca^{2+} was completely chelated by the BAPTA administration. Since the thrombin-mediated intracellular Ca^{2+} rise includes a Ca^{2+} influx component as well as Ca^{2+} mobilization from intracellular sources (Sage and Rink, 1986), the BAPTA was an effective chelator for both sources of Ca^{2+}. Serotonin uptake in platelets treated with genistein (100 μM, 37°C for 15 min) was 40 ± 5% of control (mean ± S.E.M., $n = 5$, $P < 0.001$, paired T-test); uptake in BAPTA-AM-treated platelets (10 μM, 37°C for 30 min) was 44 ± 14% of control ($n = 5$, $P < 0.025$, paired T-test). When genistein was administered to BAPTA-AM-treated platelets, uptake dropped to 25 ± 6% of vehicle-treated controls ($n = 5$, $P < 0.001$, paired T-test). Uptake after the genistein-BAPTA combination was 61 ± 5% ($P < 0.005$) compared to BAPTA-AM treatment alone.

This result suggests that mobilizable Ca^{2+}, as chelated by BAPTA, is not required for genistein-mediated inhibition of serotonin uptake in platelets. A more direct, non-Ca^{2+} mediated effect of tyrosine kinase inhibitors on uptake (i.e., via phosphorylation changes) is consistent with these results. It is unlikely that inhibition of serotonin uptake by tyrosine kinase inhibitors is due to release of endogenous serotonin stores, since genistein is actually known to inhibit rather than enhance serotonin release (IC_{50} ~ 54 μM) (Murphy et al., 1993). Incubation of platelets with the tyrosine kinase inhibitor, herbimycin A (10 μM, 15 min, 37°C) (from *Streptomyces hygroscopicus*, Sigma Chem. Co., St. Louis, MO, USA), also rapidly decreases serotonin uptake (296% ctrl., mean ± S.E.M., $n = 3$, $P < 0.01$, paired T-test), indicating that structurally dissimilar tyrosine kinase inhibitors (Levitzki and Gazit, 1995) are all able to inhibit serotonin uptake. The data with methyl 2,5-dihydroxycinnamate also indicate that the inhibition of uptake requires the continuous presence of inhibitor since the reversion to control levels of uptake closely follow the stability curve of the inhibitor.

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