ELSEVIER

Biochimica et Biophysica Acta 1283 (1996) 14–20

BBA Biochimica et Biophysica Acta

Adenosine diphosphate inhibits the serotonin transporter

George M. Anderson [a,b,*], Laura M. Hall [a], William C. Horne [c], Jing Xia Yang [a]

[a] The Child Study Center, Yale University School of Medicine, Box 207900, New Haven, CT 06520-7900, USA
[b] Department of Laboratory Medicine, Yale University School of Medicine, New Haven, CT 06510, USA
[c] Department of Cell Biology, Yale University School of Medicine, New Haven, CT 06510, USA

Received 26 December 1995; accepted 1 April 1996

Abstract

Adenosine 5'-diphosphate (ADP) caused rapid and significant reductions in the rates of [^{3}H]serotonin uptake observed for human platelets, human platelet vesicles, and rat brain synaptic vesicles. Estimated V_{max} values in platelets ($N = 15$), platelet vesicles ($N = 3$), and synaptic vesicles ($N = 3$) exposed to 100 μM ADP were $42.3 \pm 11.4\%$, $78.8 \pm 1.4\%$, and $56.8 \pm 9.9\%$ of control values, respectively. The EC_{50} values observed for ADP in platelets and platelet vesicles were 10–24 μM. Exposure to 100 μM ADP had small, inconsistent effects on K_M values observed for the platelet transporter. ADP (100 μM) caused only a slight competitive inhibition of the platelet membrane binding of [^{3}H]citalopram, a ligand for the 5HT uptake site of the transporter (5.0% displacement of 1.0 nM [^{3}H]citalopram, 13% increase in apparent K_D).

The ADP analogue 2-methylthioADP caused similar decreases in the rates of platelet [^{3}H]serotonin uptake, while a number of other related compounds had little or no effect on rates of platelet uptake. The ADP-effect on uptake was rapid, occurring in less than 2.5 s, and was additive with reductions produced by protein kinase C (PKC) activation. The ADP-induced decreases in uptake did not appear to occur through the ADP receptor or known platelet second messenger systems. The exact mechanism of the ADP-effect and its functional significance remain to be determined.

Keywords: Serotonin; Serotonin transporter; Serotonin uptake; Adenosine diphosphate; ADP; Platelet

1. Introduction

The widespread use of pharmacological agents which block monoamine neurotransmitter uptake is a testament to their potent behavioral effects and a reflection of the critical role of the uptake process in central neurotransmission. Recent research has elucidated primary and secondary structures of the plasma membrane serotonin (5HT), dopamine, and norepinephrine transporters, and has increased our understanding of their function [1–5].

It is of considerable interest to determine whether monoamine uptake is physiologically regulated, and to ascertain the mechanism and extent of the possible regulation. It is clear that most transport systems [6], including those for sugars [7,8], various anions and cations [9,10], and amino acids [11], are highly regulated. Regulation of these systems is often receptor-mediated and can occur both through rapid conformational/chemical modifications and longer-term changes in levels of transporter expression.

There has been a continuing interest in the regulation of monoamine uptake [12–20]. Research in this area has increased recently with reports of short-term regulation of 5HT uptake after protein kinase C activation [21,22], calmodulin inhibition [23], ethanol treatment [24], and adenosine receptor stimulation [25]. Long-term regulation through altered levels of transporter expression has been reported after protein kinase A activation [26–28] and following the chronic administration of antidepressants [29–32] and estradiol [16]. In preliminary studies we have reported on a rapid, marked, decrease in platelet 5HT uptake after exposure to adenosine 5'-diphosphate (ADP) [33,34]. Reported here are studies characterizing the ADP-effect on 5HT uptake in human platelets, platelet vesicles, and rat brain synaptic vesicles, and examining the mechanism of the effect in platelets.

* Corresponding author. Yale Child Study Center, P.O. Box 207900, New Haven, CT 06520-7900, USA. Fax: +1 203 7857611.

PII S0005-2736(96)00073-9

2. Experimental procedures

2.1. Materials

Sodium nitroprusside, epinephrine, serotonin, desimpramine, staurosporine, mezerein, and adenosine diphosphate (ADP) and related compounds were purchased from Sigma (St. Louis, MO). The compounds 2-methylthioadenosine diphosphate (2MeSADP), 2-methylthioadenosine triphosphate (2MeSATP), and 8-bromo-cyclic guanosine monophosphate (8-BrcGMP) were obtained from RBI (Natick, MA). [^{3}H]Serotonin ([^{3}H]5HT) creatinine sulfate was purchased from Amersham (Arlington Heights, IL), [^{3}H]citalopram was purchased from New England Nuclear (Boston, MA). Fluoxetine was a gift of the Eli Lilly Co.

2.2. Platelet [^{3}H]5HT uptake

Blood from normal, healthy, unmedicated, adult volunteers was drawn into syringes containing (9:1, v/v) acid citrate-dextrose (ACD, NIHA). After centrifugation for 10 min at $190 \times g$ using a swinging-bucket rotor, the platelet-rich plasma (PRP) was drawn off and the erythrocyte layer centrifuged for 10 min at $1200 \times g$ to prepare platelet-poor-plasma (PPP). The platelet concentration of the PRP was adjusted to $2.13 \cdot 10^8$/ml with the autologous PPP and further diluted with 9 vols. of platelet uptake buffer (calcium-free pH 7.4 Krebs–Henseleit phosphate buffer; 6.92 g NaCl, 0.35 g KCl, 0.29 g $MgSO_4 \cdot 7H_2O$, 0.16 g KH_2PO_4, 2.1 g $NaHCO_3$, 2.1 g glucose per liter). The diluted platelets were maintained at 37°C prior to use in the 5HT uptake assay. Platelet [^{3}H]5HT uptake assays were performed in siliconized 12 × 75-mm glass tubes in a final volume of 0.5 ml containing 10^7 platelets. Platelets were preincubated at 37°C with the various agents for specified times prior to the addition of [^{3}H]5HT. The incubation was continued 1 min after the addition of [^{3}H]5HT, at which time uptake was terminated by diluting the sample with 4 ml of ice-cold platelet uptake buffer and rapid filtration through GF/C glass-fiber filters (Whatman). The tubes and filters were washed twice with 4-ml vols. of cold buffer, and the collected [^{3}H]5HT determined by liquid scintillation counting. Each point was determined in triplicate. Non-specific incorporation was determined by including the 5HT uptake inhibitor fluoxetine (10 μM). Values for V_{max} and K_M were calculated by the method of Eadie and Hofstee [35]. In most experiments, the V_{max} was assumed to be equal to the uptake observed at 1 μM 5HT.

2.3. Platelet vesicle [^{3}H]5HT uptake

Platelet membrane vesicles, prepared by hypotonic lysis of outdated human platelet concentrates as previously described [35], were kindly supplied by Dr. Gary Rudnick. The platelet vesicles (26 mg/ml) were stored at −70°C until assayed for uptake. Uptake was determined after diluting the thawed vesicle suspension 10-fold with pH 6.7, 10 mM K_3PO_4 buffer containing 133 mM K_2SO_4 and 1 mM $MgSO_4$ (final protein concn. 2.6 mg/ml). After a 30 min incubation at 37°C, 15 or 20 μl of the diluted K^+-loaded vesicle suspension was added to platelet vesicle uptake buffer (pH 6.7, 10 mM Li_3PO_4 containing 200 mM NaCl and 1 mM $MgSO_4$) and uptake initiated by adding appropriate amounts of [^{3}H]5HT (final total volume 1.0 ml). Uptake was allowed to proceed for 2 min (37°C) and was terminated by diluting with ice-cold 212 mM NaCl, the vesicles were then collected by rapid filtration on nitrocellulose filters (0.5 μm pore size, Whatman) [36].

2.4. Rat brain synaptic vesicle [^{3}H]5HT uptake

Synaptic vesicles were prepared by manually homogenizing whole rat brain (less cerebellum) in 15 volumes (v/w) of ice-cold 0.32 M sucrose using a Kontes-Duall tissue grinder (PTFE-glass, 0.10–0.015 clearance). The homogenate was centrifuged 10 min at $1000 \times g$ (0–4°C), the supernate then centrifuged at $20\,000 \times g$ to obtain a 'P2' pellet. The pellet was resuspended in 20 volumes (vols./original wet weight) of 0.32 M sucrose and kept on ice; vesicles were not assayed for uptake until at least 20 min following resuspension. Vesicle uptake was assayed as for platelet uptake except that 100 μl of vesicle suspension was added to the platelet uptake buffer (final total volume 1.0 ml) at least 5 min before initiating uptake by the addition of [^{3}H]5HT; an uptake period of 2 min was used.

2.5. Platelet membrane [^{3}H]citalopram binding

Platelet membranes were prepared from outdated platelet concentrate (American Red Cross) as previously described [37]. [^{3}H]Citalopram (81 μCi/mmol) binding was assayed in a manner similar to that previously used to determine platelet [^{3}H]imipramine binding [37]. Studies were performed in a total volume of 300 μl, saturation studies used concentrations of [^{3}H]citalopram of from 0.25 to 4.0 nM with 10 μM fluoxetine added to define non-specific binding. Displacement experiments were performed using 1.0 or 1.5 nM [^{3}H]citalopram.

All data are expressed as mean ± standard deviation (S.D.).

3. Results

3.1. Effect of ADP and 2MeSADP on platelet [^{3}H]5HT uptake

As seen in Fig. 1, exposure of platelets to 20 or 100 μM ADP during the 1 min uptake period caused marked decreases in the rate (V_{max}) of platelet [^{3}H]5HT transport, with no consistent effect on K_M. The effect was near maximal at 100 μM ADP (see Fig. 2a), with an average

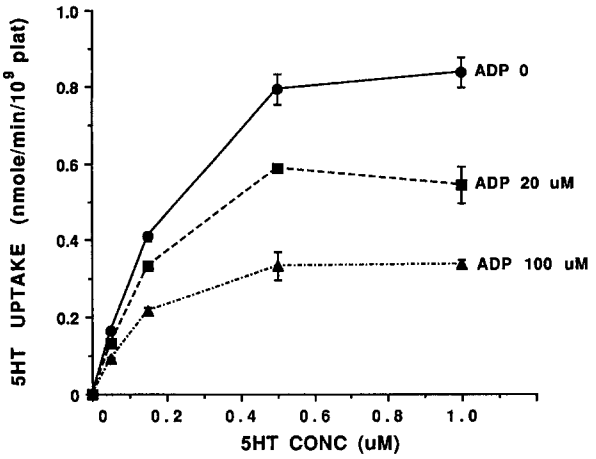

Fig. 1. Effect of ADP on platelet [^{3}H]5HT uptake. Platelets were exposed to 0, 20, or 100 μM ADP during a 1 min uptake period, using 5HT concentrations ranging from 0.05 to 1.0 μM. Experiments were performed with a 1 min uptake period, at 37°C. Uptake is expressed as specific incorporation per 10^9 platelets (mean ± S.D. of triplicate samples). Similar results were obtained in a second saturation experiment.

(±S.D.) decrease in V_{max} to 42.3 ± 11.4% ($N = 15$) of control values (V_{max} estimated from uptake at 1.0 μM). An EC_{50} of 24 ± 15 μM ADP was observed ($N = 5$). As shown in Fig. 2b, similar decreases in the estimated V_{max} (determined from the uptake rate observed at 1 μM 5HT) were seen when platelets were exposed to the hydrolysis-resistant ADP analogue 2MeSADP during the uptake period. On average, the estimated V_{max} decreased to 55.9 ± 2.1% ($N = 3$) of control in the presence of 10 μM 2MeSADP. The effect was maximal at 10 μM 2MeSADP, EC_{50} values for 2MeSADP ranged from 0.4 to 4.0 μM (mean 1.7 μM).

3.2. Temporal aspects of the ADP-induced decrease in platelet [^{3}H]5HT uptake

Initial experiments revealed that no preincubation period was necessary for maximal inhibition of [^{3}H]5HT uptake by ADP. However, because platelets were exposed to ADP throughout the 1 min uptake period it was unclear just how rapidly the ADP effects were manifest. When uptake was measured in uptake periods ranging from 2.5 to 60 s, a significant ($P < 0.05$) decrease was seen even for the briefest exposure/uptake time of 2.5 s (see Fig. 3).

3.3. Effect of platelet activators on platelet [^{3}H]5HT uptake

Because ADP is known to cause an inhibition of platelet adenylate cyclase and an increase in platelet Ca^{2+} concentration [38], we examined the effects of epinephrine (EPI, an inhibitor of platelet adenylate cyclase), of the calcium ionophore A23187, and of the two agents in combination. Five min preincubation with either 10 μM EPI, 100 μM EPI, 1 μM A23187, 10 μM A23187, or a combination of

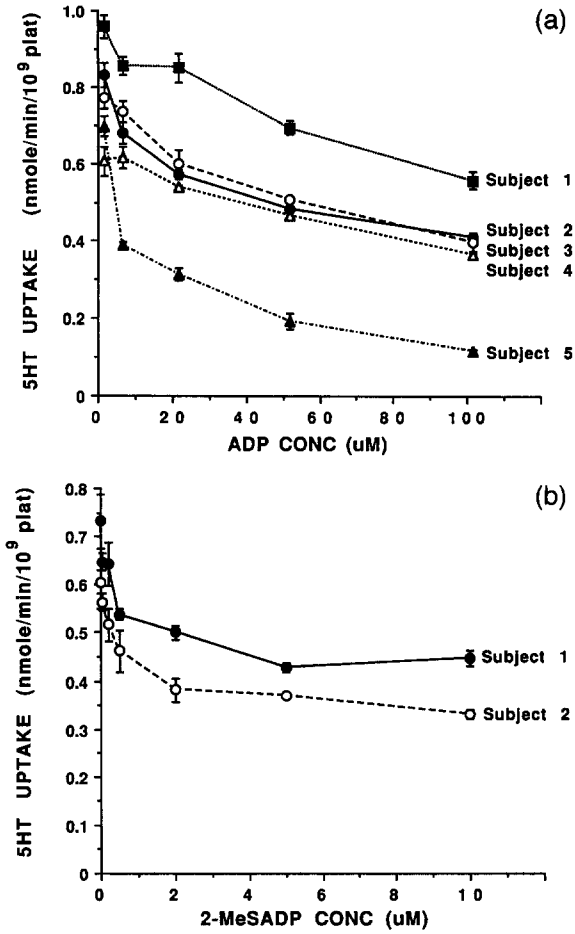

Fig. 2. Concentration dependence of ADP and 2MeSADP effects on platelet [^{3}H]5HT uptake. (a) Platelet 5HT uptake observed in five different subjects. Uptake was carried out in the presence of 0, 1, 5, 20, 50 or 100 μM ADP. (b) Platelet 5HT uptake observed in two different subjects. Platelets were exposed to 0, 0.05, 0.1, 0.5, 2.0, 5.0 and 10 μM 2MeSADP. Experiments were performed with 1 μM 5HT, 1 min uptake period, at 37°C.

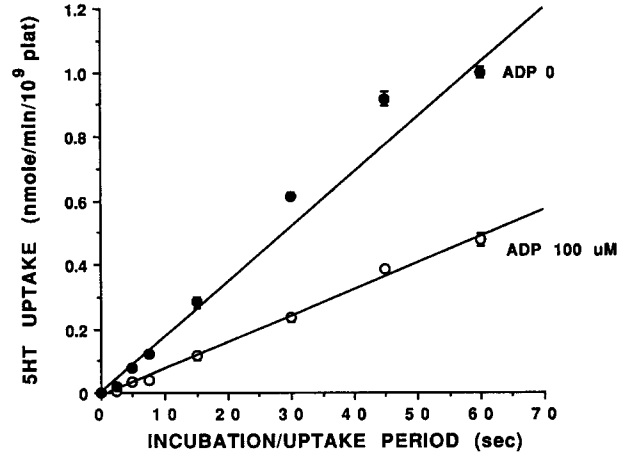

Fig. 3. Time dependence of the ADP effect on platelet [^{3}H]5HT uptake. Platelet [^{3}H]5HT uptake was measured for a range of uptake periods ranging from 2.5 to 60 s. Uptake was determined using 1 μM 5HT in the presence and absence of 100 μM ADP. Significant reductions were seen for all uptake periods examined ($P = 0.001$ for 5–60 s uptake periods, $P = 0.05$ for 2.5 s, according to Students' t-test.

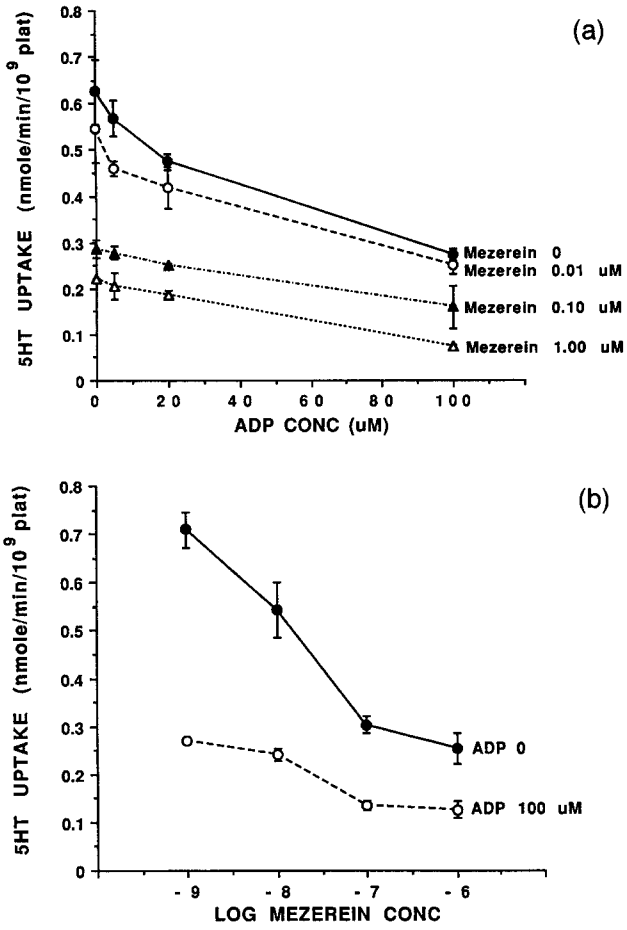

Fig. 4. Effect of the combination of a PKC activator and ADP on platelet [^{3}H]5HT uptake. (a) Platelets were preincubated (20 min) with the indicated concentrations of mezerein, uptake (1.0 μM 5HT, 1 min) was then carried out in the presence of 5–100 μM ADP. (b) Platelets were preincubated (20 min) with a range of mezerein concentrations (1 nM–1 μM), uptake (1.0 μM 5HT, 1 min) was then measured in the presence (100 μM) or absence of ADP. Values are means (±S.D.).

10 μM EPI and 10 μM A23187 had little or no (< 5% reduction) effect on the rate of platelet [^{3}H]5HT uptake. Preexposure to the nitric oxide source sodium nitroprusside (2–50 μM) had no effect on basal platelet [^{3}H]5HT uptake, and was without effect on the reduction in uptake rate caused by 100 μM ADP (data not shown).

3.4. Effects of protein kinase modulation on platelet [^{3}H]5HT uptake

In order to study the relationship of the ADP-mediated effect to the previously described down-regulation of platelet uptake caused by protein kinase C (PKC) activators [21,22], we examined the effects of the PKC activator mezerein in combination with either ADP or 2MeSADP. As shown in Fig. 4a the effects of mezerein and ADP were additive, with a range of mezerein concentrations causing relatively constant additional percentage declines in [^{3}H]5HT uptake regardless of the extent of ADP-induced inhibition. Similar additive effects were seen when dose–response curves for mezerein with or without ADP (100 μM) were obtained (Fig. 4b). The PKC inhibitor staurosporine, shown previously to block the inhibitory effects of PKC activation on [^{3}H]5HT uptake [22], at concentrations of 0.1–10 μM had no apparent effect on the down-regulation produced by 100 μM ADP. The possible involvement of cyclic guanosine monophosphate (cGMP)-dependent protein kinase (PKG) in the regulation of platelet 5HT uptake was examined by pretreatment with the cell-permeant cGMP analogue 8-bromo-cGMP. No effect on uptake was seen over a concentration range of 1 μM to 2 mM, with pretreatment times of up to 20 min (data not shown).

3.5. Effects of ADP-related compounds on platelet [^{3}H]5HT uptake

A range of purine derivatives and other ADP-related compounds, including adenine, adenosine, 2-chloroadenosine, adenosine monophosphate (AMP), cyclic AMP, adenosine triphosphate (ATP), 2MeSATP, guanosine monophosphate, guanosine diphosphate, guanosine triphosphate, theophylline, theobromine, and caffeine, were tested for their possible effect on platelet [^{3}H]5HT uptake (concn. of agents 0.1 or 1 mM, 0.15 μM 5HT, 1 min uptake period). Uptake rates observed in the presence of all but one of the compounds tested ranged from 92.3 to 108% of control, and were not significantly different from control. However, 2MeSATP did significantly reduce uptake to 76.8–82.2% of the control value (P values 0.0001 and 0.03). In the same series of experiments 100 μM ADP reduced uptake to 56.3 ± 6.8% ($N = 3$) of control.

3.6. Effect of ADP on platelet vesicle [^{3}H]5HT uptake

As seen in Fig. 5a, exposure of platelet vesicles to ADP during the 2 min uptake period significantly decreased the rate of [^{3}H]5HT uptake. Uptake at 1.0 μM 5HT averaged 78.8 ± 1.4% ($N = 3$) of control in platelets exposed to 100 μM ADP. There was little effect of ADP on the affinity (K_M) of the transporter for 5HT. An apparent EC_{50} of 10 μM was observed for the ADP-induced reduction in the rate of platelet vesicle 5HT uptake.

3.7. Effect of ADP on rat brain synaptic vesicle [^{3}H]5HT uptake

Synaptic vesicles exposed to ADP during the 2 min uptake period showed significantly lower rates of [^{3}H]5HT uptake compared to controls (see Fig. 5b). Synaptosomal uptake observed at 1.0 μM 5HT was 56.8 ± 9.9% ($N = 3$) of controls in the presence of 100 μM ADP. Similar reductions were seen in the V_{max} values calculated from Eadie–Hofstee analysis; with only small increases in calculated K_M values. An apparent EC_{50} of 15 μM was

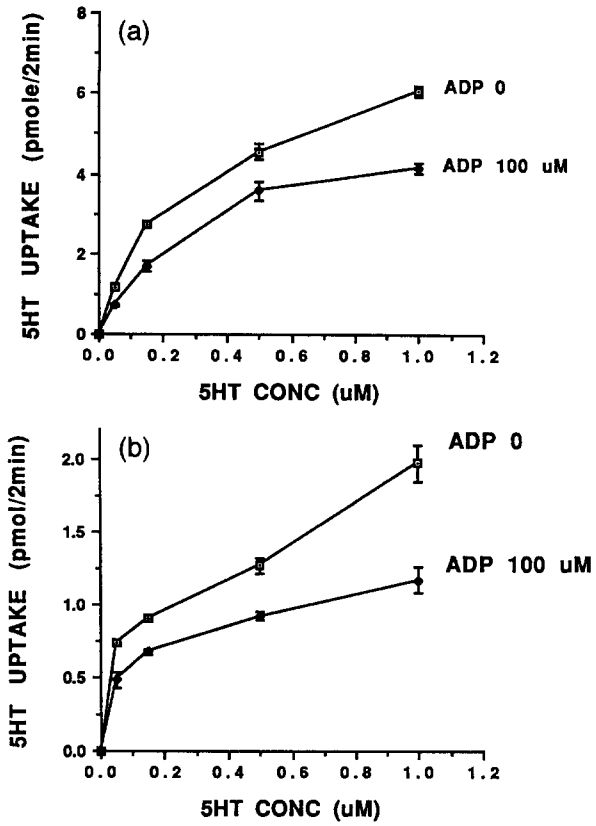

Fig. 5. Effect of ADP on platelet and synaptic vesicle [^{3}H]5HT uptake. (a) Platelet vesicles were exposed to 0 or 100 μM ADP during a 2 min uptake period, 5HT concentrations ranged from 0.05 to 1.0 μM. Uptake is expressed as specific (not fluoxetine blocked) incorporation per 2 min per tube (50 μg vesicle protein per tube). Graph is representative of two saturation experiments carried out with platelet vesicles. (b) Synaptic vesicles were exposed to 0 or 100 μM ADP during a 2 min uptake period. Specific uptake was expressed as incorporation per 2 min per tube (P2 fraction equivalent to 5 mg original wet weight brain per tube). Graph is representative of two saturation experiments carried out with synaptic vesicles.

observed for ADP. Similar effects of ADP on the rate of 5HT uptake were observed when 2 mM Ca^{2+} was added to the uptake buffer (data not shown).

3.8. Effect of ADP on platelet membrane [^{3}H]citalopram binding

Exposure of platelet membranes to 100 μM ADP during the 60 min (22°C) incubation period caused a slight increase (+13%) in the apparent K_D for [^{3}H]citalopram and was without effect on the observed B_{max}. In displacement experiments, 100 μM ADP caused only a 5.0% inhibition of 1.0 nM [^{3}H]citalopram binding. In contrast, nearly complete displacement of [^{3}H]citalopram was seen with fluoxetine (IC_{50} = 4.0 nM) and desipramine (IC_{50} = 71 nM).

Due to the potential for metabolism of ADP over the 60 min incubation period, binding experiments were also carried out using the hydrolysis-resistant 2MeSADP and with shorter incubation times. The addition of ADP (10 and 100 μM) during shorter term incubations (non-equilibrium conditions: 5 and 10 min, 22°C) of platelet membranes with [^{3}H]citalopram had little or no effect on binding (< 5% displacement). Similarly, the presence of 2MeSADP (1.0 and 10 μM) failed to appreciably affect the binding of [^{3}H]citalopram seen after a 60 min incubation (data not shown). In studies on the effect of increasing serotonin concentrations (0.05 to 2.5 μM) on [^{3}H]citalopram binding to platelet membranes, ADP (100 μM) and 2MeSADP (10 μM) did not influence the displacement of [^{3}H]citalopram by serotonin (data not shown).

4. Discussion

ADP is a well-studied platelet activator, causing shape change, aggregation, calcium influx and inhibition of adenylate cyclase [38]. The reduction in platelet 5HT uptake does not appear to be due to these secondary effects of ADP. Studies were done in non-aggregating, non-releasing, conditions (diluted PRP, low Ca^{2+}) and several agents which cause platelet shape change (including 5HT itself) did not cause a reduction in uptake.

The possible involvement of Ca^{2+}, cyclic adenosine monophosphate (cAMP), and other second messenger systems was studied; Ca^{2+} and cAMP were of particular interest given ADP's effects on both these intracellular signals. The calcium ionophore A23187 failed to affect [^{3}H]5HT uptake at concentrations and pretreatment times known to cause substantial increases in intracellular Ca^{2+}. Epinephrine, at concentrations reported to markedly inhibit platelet adenylate cyclase activity, also had no effect on uptake rates. It should be noted that in previous experiments we have not observed any change in uptake after exposure to dibutyryl cAMP, an activator of protein kinase A (unpublished data), and that the reported effects of PKA activation on uptake in a placental cell line occurred after 4–8 h of exposure to PKA activators [26,27]. When EPI and A23187 were combined in an attempt to duplicate the known second-messenger effects of ADP, there was still no effect on platelet [^{3}H]5HT uptake rates.

Activation of PKC has been reported to decrease the rate of 5HT transport in several cell types [21,22,25]. The possible mechanisms of this effect have been explored [22] and site-directed mutagenesis experiments have begun to localize possible sites of PKC regulation [39,40]. We were therefore quite interested in studying the relationship of the PKC-induced down-regulation to the ADP-mediated effect. In studies combining the PKC activator mezerein with either ADP or 2MeSADP, the effects appeared to be additive. The lack of an effect of the PKC inhibitor staurosporine on the ADP-mediated decrease in uptake provides additional evidence supporting the idea that PKC activators and ADP are acting to decrease [^{3}H]5HT uptake through distinct and independent processes.

In experiments examining the effect of the cGMP-dependent protein kinase (PKG) activator, 8-BrcGMP, over a range of concentrations and treatment times, we found virtually no change in platelet [^{3}H]5HT uptake. As mentioned, PKA activation also did not alter platelet [^{3}H]5HT uptake or influence the ADP effect. The PKG findings contrast with the reported increase of approximately 20% in [^{3}H]5HT uptake observed for rat basophilic leukemia (RBL) cells after a 15 min treatment with 10 μM 8-BrcGMP [25]. Although further research is necessary to establish the dose–response characteristics of 8-BrcGMP in RBL cells, it appears that the regulation of the transporter in platelets and RBL cells may be dissimilar in this respect.

Recent reports of an effect of nitric oxide on 5HT transport in RBL cells [25] and on dopamine transport in rat striatal synaptosomes [41] stand in contrast to our findings of unchanged platelet [^{3}H]5HT uptake after preincubation with sodium nitroprusside. The observed lack of an effect of sodium nitroprusside on the platelet V_{max} and on the ADP-induced reduction in V_{max} strongly suggests that nitric oxide does not regulate or mediate ADP-regulation of the platelet 5HT transporter.

The idea that the ADP effect does not proceed through the ADP receptor or second messenger systems is strongly supported by the results obtained using platelet vesicles and rat brain synaptic vesicles. There is a robust ADP effect on 5HT uptake in both types of vesicles, despite the virtual elimination of intracellular and extracellular Ca^{2+} in the vesicle preparations. Other second messenger systems would also be expected to be eliminated or severely curtailed in the vesicle systems. In addition, there is little reason to believe that the ADP receptor and the serotonin transporter are colocalized on neurons of the central nervous system. The demonstration of the ADP effect in synaptic vesicles is, therefore, further evidence against the effect being mediated through the ADP receptor.

Although ADP appears to reduce the rate of platelet [^{3}H]5HT uptake by acting at the transporter, it produces only small decreases in the apparent affinity of [^{3}H]5HT for the transporter and it has only a small inhibitory effect on [^{3}H]citalopram binding to the transporter. This similarity with respect to 5HT and citalopram is expected as thay are both considered to bind, in a largly overlapping manner, to the uptake site of the transporter [42]. Further evidence that ADP does not act by altering 5HT binding comes from the data showing no effect of ADP on the displacement of [^{3}H]citalopram by 5HT. Thus, while it seems likely that ADP acts directly on the transporter, it appears to act at a site which is largely distinct from the uptake site itself and it does not act through an alteration in the initial binding of 5HT. Rather, it may be that ADP acts to alter some aspect of the subsequent translocation process.

The data serve to raise several issues for consideration. First, one can speculate that in certain circumstances inhibition of platelet 5HT uptake by ADP may lead to increased 5HT-mediated platelet activation and recruitment. There is also the possibility that, if the plasma membrane 5HT transporter were to be incorporated into the granule membrane in an outward-directed fashion (for instance, after granule fusion and recycling) granular ADP would serve to inhibit the misplaced (adventitious) transporter. This might limit potentially harmful reductions in granular 5HT and increases in cytosolic 5HT.

A second issue concerns the general area of non-competitive inhibition of the 5HT transporter. It appears that, if ADP does directly interact with the transporter, it does not bind to sites which overlap substantially with the 5HT uptake site itself. This is in direct contrast to the large family of selective and non-selective competitive serotonin reuptake inhibitors. Instead, ADP could be considered to have the properties of an encoid or regulatory agent. Much of the previous speculation along these lines focused on the possibility of non-competitive regulation through the 'imipramine binding site'. It is now clear that the imipramine binding site is the same as, or largely overlapping with, the 5HT uptake site [43,44]. However the ADP data indicate that it may be possible to have an agent that interacts with the transporter without substantially overlapping with the uptake site.

To summarize our findings regarding the effects of ADP on platelet 5HT uptake: The ADP-mediated effect is rapid and predominantly noncompetitive, appears not to proceed through second messenger systems or the ADP receptor, and is additive with the previously established PKC effect on uptake. The functional significance and precise mechanism of the ADP-effect remain to be determined.

Acknowledgements

We gratefully acknowledge the support of the National Institute of Health (MH30929) and Korczak Stichting (Foundation). We thank Ms. Ilaria Borghese for her assistance with graphing and data analysis.

References

[1] Blakely, R.D., Berson, H.E., Fremeau, R.T., Jr., Caron, M.G., Peek, M.M., Prince, H.K. and Bradley, C.C. (1991) Nature 354, 66–70.
[2] Hoffman, B.J., Mezey, E. and Brownstein, M.J. (1991) Science 254, 579–580.
[3] Rudnick, G. and Clark, J. (1993) Biochim. Biophys. Acta 1144, 249–263.
[4] Brownstein, M.J. and Hoffman, B.J. (1994) Rec. Prog. Hormone Res. 49, 27–42.
[5] Worrall, D.M. and Williams, D.C. (1994) Biochem. J. 297, 425–436.
[6] Pardee, A.B. and Palmer, C.M. (1973) in Rate control of biological processes (Doris, D.P., ed.), pp. 133–144, Cambridge University Press, New York.

[7] Rozen, O.M. (1987) Science 237, 1452–1458.
[8] Joost, H.G. and Weber, T.M. (1989) Diabetologia 32, 831–838.
[9] Siegelbaum, S.A., Camerdo, J.S. and Kandet, E.R. (1982) Nature 299, 413–417.
[10] Brown, D.R., Chandon, R., Quito, F.L. and Seybold, V.S. (1988) Life Sci. 43, 2193–2201.
[11] Souba, W.W. and Pacitti, A.J. (1992) J. Parent Ent. Nutr. 16, 569–578.
[12] Roach, M.K., Davis, D.L., Pennington, W. and Nordyke, E. (1973) Life Sci. 12, 433–441.
[13] Boyd, F.T., Clark, D.W., Muther, T.F. and Raizanda, M.K. (1985) J. Biol. Chem. 260, 15880–15884.
[14] Hendley, E.D., Whittemore, S.R., Chaffee, J.E. and Ehrlich, Y.H. (1988) J. Neurochem. 50, 263–273.
[15] Tukiainen, E. (1981) Acta Pharmacol. Toxicol. 48, 139–144.
[16] Rehavi, M., Sepcuti, H. and Weizman, A. (1987) Brain Res. 410, 135–139.
[17] Lee, R.H.K. and Chan, M.Y. (1984) Clin. Exp. Pharmacol. Physiol. 11, 53–59.
[18] Rausch, J.L., Fefferman, M., Ladisich, D.G. and Menard, M. (1989) Prog. Neuropsychopharmacol. Biol. Psychiatr. 13, 907–916.
[19] Bosin, T.R. (1989) J. Pharmacol. Exp. Ther. 248, 67–72.
[20] Barbaccia, M.C. and Costa, E. (1988) in Perspectives in Pharmacology: A Collection in Honor of Earl Usdin, pp. 333–349, Alan R. Liss, New York.
[21] Myers, C.L., Lazo, J.S. and Pitt, B.R. (1989) Am. J. Physiol. 257, L253–L259.
[22] Anderson, G.M. and Horne, W.C. (1992) Biochim. Biophys. Acta 1137, 331–337.
[23] Jayanthi, L.D., Ramamoorthy, S., Mahesh, V.B., Leibach, F.H. and Ganapathy, V. (1994) J. Biol. Chem. 269, 14424–14429.
[24] Alexi, T. and Azmitia, E.C. (1991) Brain Res. 544, 243–247.
[25] Miller, K.J. and Hoffman, B.J. (1994) J. Biol. Chem. 269, 27351–27356.
[26] Cool, D.R., Leibach, F.H., Bhalla, V.K., Mahesh, V.B. and Ganapathy, V. (1991) J. Biol. Chem. 266, 15750–15757.
[27] Ramamoorthy, S., Cool, D.R., Mahesh, V.B., Leibach, F.H., Melikian, H.E., Blakely, R.D. and Ganapathy, V. (1993) J. Biol. Chem. 268, 21628–21631.
[28] King, S.C., Tiller, A.A., Chang, A.S. and Lam, D.M. (1992) Biochem. Biophys. Res. Commun. 183, 487–491.
[29] Lesch, K.P., Aulakh, C.S., Wolozin, B.L., Tolliver, T.J., Hill, J.L. and Murphey, D.L. (1993) Mol. Brain Res. 17, 31–35.
[30] Hrdina, P.V. (1987) Eur. J. Pharmacol. 138, 159–168.
[31] Pineyro, G., Blier, P., Dennis, T., De Montigny, C. (1994) J. Neurosci. 14, 3036–3047.
[32] Lopez, J.F., Chalmers, D.T., Vazquez, D.M., Watson, S.J. and Akil, H. (1994) Biol. Psychiatr. 35, 287–290.
[33] Anderson, G.M., Hall, L.M. and Yang, J.X. (1993) Soc. Neurosci. Abstr., p. 936.
[34] Anderson, G.M. and Hall, L.M. (1994) Soc. Neurosci. Abstr., p. 294.
[35] Hofstee, B.H.J. (1952) Science 116, 329.
[36] Rudnick, G. and Nelson, P. (1978) Biochemistry 17, 5300–5303.
[37] Anderson, G.M., Minderaa, R.M., Van Benthem, P.P., Volkmar, F.R. and Cohen, D.J. (1984) Psychiatr. Res. 11, 133–141.
[38] Siess, W. (1989) Physiol. Rev. 69, 58–178.
[39] Hoffman, B.J. and Miller, K.J. (1994) Soc. Neurosci. Abstr., p. 627.
[40] Oian, Y., Melikan, H.E., Moore, K.R., Duke, B.J. and Blakely, R.D. (1995) Soc. Neurosci. Abstr., p. 865.
[41] Pogren, S., Baumann, M.H. and Kuhar, M.J. (1994) Brain Res. 641, 83–91.
[42] D'Amato, R.J., Largent, B.L., Snowman, A.M. and Snyder, S.H (1987) J. Pharmacol. Exp. Ther. 242, 364–371.
[43] Humpreys, C.J., Wall, S.C. and Rudnick, G. (1994) Biochemistry 33, 9118–9125.
[44] Barker, E.L., Kimmel, H.L. and Blakely, R.D. (1994) Mol. Pharmacol. 46, 799–807.