Temporal Regulation of Agonist Efficacy at 5-Hydroxytryptamine (5-HT)\textsubscript{1A} and 5-HT\textsubscript{1B} Receptors

KELLY A. BERG, KENDA L. J. EVANS,1 JODIE D. CROPPER, and WILLIAM P. CLARKE

Department of Pharmacology, University of Texas Health Science Center, San Antonio, Texas

Received August 6, 2002; accepted August 30, 2002

ABSTRACT
Coactivation of purinergic (P\textsubscript{2Y}) receptors reduces agonist efficacy at serotonin\textsubscript{1A} (5-HT\textsubscript{1A}), but not 5-HT\textsubscript{1B} receptors. Herein, we report that pretreatment for 5 min with the P\textsubscript{2Y} receptor agonist ATP reduced agonist responsiveness at the 5-HT\textsubscript{1A}, but not at the 5-HT\textsubscript{1B}, receptor. The effect of ATP pretreatment on the 5-HT\textsubscript{1A} receptor response rapidly reversed within a 10 min time frame between P\textsubscript{2Y} receptor and 5-HT\textsubscript{1A} receptor activation. ATP pretreatment effects on 5-HT\textsubscript{1A} agonist responsiveness were blocked by the protein kinase inhibitors staurosporine and bisindolylmaleimide, suggesting that the ATP-mediated temporal regulation involves activation of protein kinase C (PKC). Moreover, the temporal effect of ATP was blocked by incubation with 1% ethanol, suggesting that consequences of phospholipase D (PLD) activation play a role. ATP pretreatment blocked the inhibitory effect produced by 5-HT\textsubscript{2C} receptor activation on the 5-HT\textsubscript{1A}, but not the 5-HT\textsubscript{1B}, receptor response, suggesting that the 5-HT\textsubscript{1A} receptor itself was the target for PLD/PKC action. Finally, ethanol did not block the reduction in responsiveness of the 5-HT\textsubscript{1A} receptor system produced by activation of PKC with phorbol ester treatment, suggesting that PKC activation lies downstream of PLD. Taken together, these data suggest that activation of P\textsubscript{2Y} receptors can reduce responsiveness of the 5-HT\textsubscript{1A} receptor system via a PLD/PKC-dependent mechanism that is highly dependent upon the temporal pattern of receptor activation. Moreover, this work underscores the importance of time as a variable in receptor signaling cross talk and serves to further illustrate differences between the 5-HT\textsubscript{1A} and 5-HT\textsubscript{1B} receptor systems.

The serotonin (5-HT\textsubscript{1A}) and 5-HT\textsubscript{1B} receptors are seven-transmembrane spanning receptors that inhibit adenylyl cyclase activity via pertussis toxin-sensitive G proteins (i.e., Gi/Go) in brain tissue (De Vivo and Maayani, 1985; Bouhelal et al., 1988; Schoeffer and Hoyer, 1989) and heterologous expression systems (Raymond, 1991; Adham et al., 1992; Hamblin et al., 1992; Albert et al., 1996). Both 5-HT\textsubscript{1A} and 5-HT\textsubscript{1B} receptors are autoreceptors on serotonergic neurons and thus play pivotal roles in the regulation of serotonergic neurotransmission (Pineyro and Blier, 1999; Knobelman et al., 2000). Alterations in the responsiveness of 5-HT\textsubscript{1A} and 5-HT\textsubscript{1B} receptor systems, and consequently of serotonergic neurotransmission, have been implicated in the etiology of a variety of psychiatric disorders, including anxiety, depression, obsessive-compulsive disorders, schizophrenia, and eating disturbances (Lucki, 1998). Furthermore, drugs that influence serotonergic neurotransmission have proven to be valuable therapeutic agents (Blier and de Montigny, 1999). Accordingly, knowledge of how the responsiveness of the 5-HT\textsubscript{1A/B} receptor systems can be regulated may lead to better understanding of the role these receptor subtypes play in various physiological and pathological conditions and aid in the development of new clinically useful drugs.

Several reports have indicated that the responsiveness of the 5-HT\textsubscript{1A} and 5-HT\textsubscript{1B} receptor systems to agonist activation can be regulated by intracellular cross talk mechanisms coupled to heterologous receptor systems (Raymond, 1991; Harrington et al., 1994; Lembo and Albert, 1995; Hensler et al., 1996). For example, we recently found that coactivation of receptors coupled to phospholipid signaling cascades [phospholipase C (PLC) and phospholipase A\textsubscript{2} (PLA\textsubscript{2})] can alter agonist efficacy at 5-HT\textsubscript{1A} and 5-HT\textsubscript{1B} receptors (Berg et al., 1994b, 1996; Evans et al., 2001). Receptor-mediated activation of PLA\textsubscript{2} reduces the responsiveness of the 5-HT\textsubscript{1B} receptor system via a cyclooxygenase-sensitive metabolite of arachidonic acid that targets adenylyl cyclase. Consequences of

ABBREVIATIONS: 5-HT, 5-hydroxytryptamine, serotonin; PLC, phospholipase C; PLA\textsubscript{2}, phospholipase A\textsubscript{2}; PKC, protein kinase C; [Ca\textsuperscript{2+}]\textsubscript{i}, intracellular calcium concentration; BMY 7378, 8-((2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl)-8-azaspiro(4,5)decan-7,9-dione dihydrochloride; PLD, phospholipase D; dp-5-GT, dipropyl 5-carboxamidotryptamine; 5-GT, 5-carboxamidotryptamine; DOI, (z)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane; p-MPPF, 4-((2'-methoxy-)-phenyl-1-[2'-(N'-Z'-pyridyl)]-p-fluorobenz-amidoethyl-piperazine; CHO, Chinese hamster ovary; PDBu, phorbol dibutyrate.
PLC signaling (PKC and [Ca\(^{2+}\)]_\text{i}) do not alter the 5-HT\(_{1B}\) system (Berg et al., 1994b, 1996). On the other hand, the 5-HT\(_{1A}\) receptor system is more dynamically regulated. As for the 5-HT\(_{1B}\) receptor, activation of the PLA\(_2\) reduces 5-HT\(_{1A}\) receptor responsiveness; however, unlike the 5-HT\(_{1B}\) receptor, PLC-mediated increases in [Ca\(^{2+}\)]_\text{i} enhance 5-HT\(_{1A}\) agonist efficacy. The net effect of coactivation of a receptor that couples to both PLA\(_2\) and PLC depends upon the relative capacity of the receptor to produce arachidonic acid versus increase [Ca\(^{2+}\)]_\text{i} (Evans et al., 2001). Interestingly, in these experiments in which heterologous receptors were coactivated with 5-HT\(_{1A}\) receptors, there was no role for PKC activation in regulation of 5-HT\(_{1A}\) agonist efficacy. This lack of a role for PKC was surprising in that the 5-HT\(_{1A}\) receptor is a target for PKC-mediated phosphorylation, and PKC activation with phorbol esters reduces 5-HT\(_{1A}\) receptor signaling (Raymond, 1991; Harrington et al., 1994; Lembo and Albert, 1995; Hensler et al., 1996).

Time is an important variable in biological systems, however, relatively little is known about the time dependence of changes in responsiveness elicited by cross talk mechanisms between receptor systems. In this study, we explored the time course of regulation of 5-HT\(_{1A}\) and 5-HT\(_{1B}\) receptor system responsiveness in response to activation of P\(_{2Y}\) purinergic receptors. In contrast to coactivation of P\(_{2Y}\) receptors, pretreatment of cells with the P\(_{2Y}\) agonist ATP reduced responsiveness of the 5-HT\(_{1A}\), but not 5-HT\(_{1B}\), receptor system in a PKC-dependent manner. Furthermore, the data suggest that the PKC effect on 5-HT\(_{1A}\) agonist responsiveness is dependent upon upstream activation of phospholipase D (PLD). These data underscore important differences in the regulation of 5-HT\(_{1A}\) and 5-HT\(_{1B}\) receptor systems by phospholipid signaling cascades.

**Materials and Methods**

**Materials.** N,N-Dipropyl-5-carboxamidotryptamine (dp-5-CT), 5-carboxamidotryptamine (5-CT), and (\(\pm\)-1-(2,5-dimethoxy-4-iodophenyl)-2-amino-propane (DOI) were purchased from Sigma/RBI (Natick, MA); \(\text{[^{3}H]}\)-cAMP tracer was from PerkinElmer Life Sciences (Boston, MA), and anti-cAMP antibody was from ICN Pharmaceuticals (Costa Mesa, CA). Forskolin, staurosporine, and bisindolylmaleimide were purchased from Calbiochem (La Jolla, CA). Rolipram was a generous gift from Berlex Laboratories (Cedar Knolls, NJ) and 4-(2'-methoxy)-phenyl-1-[2'-(N'-2''-pyridyl)-p-fluorobenz-amido]ethyl-piperazine (p-MPPF) was a generous gift from Dr. Hank Kung (University of Pennsylvania, Philadelphia, PA). All tissue culture reagents and Hank's balanced salt solution were purchased from Invitrogen (Carlsbad, CA). All other drugs and chemicals (reagent grade) were purchased from Sigma-Aldrich (St. Louis, MO).

**Transfection and Cell Culture.** CHO-1A cells are a CHO-K1 clonal cell line that expresses stably h5-HT\(_{1A}\) receptors at a density of approx 130 fmol/mg protein as determined by BMY-7378 sensitive, \(\text{[^{3}H]}\)-(\(\pm\)-5-hydroxy-dipropylaminotetralin saturation binding (Evans et al., 2001). CHO-2C/1A cells are a clonal cell line expressing approx 200 to 250 fmol/mg protein h5-HT\(_{1A}\) receptors (Berg et al., 1994b) and which have been transfected stably to express h5-HT\(_{1A}\) receptors (~1.3 pmol/mg protein) (Evans et al., 2001). Cells were maintained in minimal essential medium-\(\alpha\) formulation supplemented with 5% fetal bovine serum and 50 \(\mu\)g/ml G418 (CHO-1A) or 125 \(\mu\)g/ml zeocin + 300 \(\mu\)g/ml hygromycin (CHO-2C/1A). For all experiments, cells were seeded into 24-well, 15-cm, or T175 tissue culture vessels at a density of 4 \(\times\) 104 cells/cm\(^2\). After 24 h, cells were washed with Hanks' balanced salt solution and placed into Dulbecco's modified Eagle's medium/F-12 (1:1) with 5 \(\mu\)g/ml insulin, 5 \(\mu\)g/ml transferrin, 30 nM selenium, 20 nM progesterone, and 100 \(\mu\)M putrescine (serum-free media) and grown for an additional 24 h before experimentation.

**Inhibition of cAMP Accumulation.** 5-HT\(_{1A}\) and 5-HT\(_{1B}\) agonist-mediated inhibition of forskolin-stimulated cAMP accumulation was determined by measuring the inhibition of cAMP accumulated in response to 1 \(\mu\)M forskolin (15 min, 37°C) in the presence of the phosphodiesterase inhibitor rolipram (0.1 mM) as described previously (Berg et al., 1994a). The selective 5-HT\(_{1A}\) receptor antagonist \(p\)-MPPF (10 \(\mu\)M; \(K_i = 1.2\) nM) was used to distinguish 5-HT\(_{1B}\) receptor responses from those mediated by 5-HT\(_{1A}\) receptors (Evans et al., 2001). Cellular cAMP content was measured by radioimmunoassay and normalized to protein content, which was measured according to the method of Lowry et al. (1951).

**Data Analysis.** For cAMP accumulation experiments, data from each experiment were normalized by defining the cAMP response to 1 \(\mu\)M forskolin as 100%. Statistical comparisons of treatment effects were done where appropriate with the Student's t test (paired). For experiments where multiple comparisons were made, one-way analysis of variance followed by Newman-Keuls post hoc test was used. Asterisks (*) denote statistically significant p values <0.05 (*), <0.01 (**), and <0.001 (***)

**Results**

CHO-K1 cells express naturally purinergic receptors of the P\(_{2Y}\) subtype (Iredale and Hill, 1993). Consistent with our previous reports (Berg et al., 1994b; Evans et al., 2001), coactivation of P\(_{2Y}\) receptors with ATP reduced the capacity of the 5-HT\(_{1B}\) receptor agonist 5-CT to inhibit forskolin-stimulated cAMP accumulation by about 40%, but did not alter agonist (dp-5-CT) responsiveness at the 5-HT\(_{1A}\) receptor system (Fig. 1). However, the action of P\(_{2Y}\) receptor activation on 5-HT\(_{1A}\) and 5-HT\(_{1B}\) receptor system responsiveness was reversed when the receptors were activated in a different temporal sequence. When cells were treated with ATP for 5 min, followed by a quick wash and further incubation...
tion without ATP for 5 min (subsequently referred to herein as "the ATP pretreatment paradigm"), agonist responsiveness at the 5-HT1A receptor system was reduced by about 55%, whereas the responsiveness of the 5-HT1B receptor system to agonist stimulation was not changed (Fig. 2). The ATP-pretreatment effect on the 5-HT1A agonist response gradually reversed as the time interval between P2Y receptor activation and the test of 5-HT1A receptor system responsiveness was increased (Fig. 2). The responsiveness of the 5-HT1B receptor system was not changed by any of the pretreatment time periods.

Coactivation of the 5-HT2C receptor expressed in CHO-2C/1A cells reduces both 5-HT1A and 5-HT1B agonist efficacy because of a greater capacity of 5-HT2C receptor activation to stimulate PLA2 versus PLC (Evans et al., 2001). As shown in Fig. 3, the ATP pretreatment paradigm blocked the reduction in 5-HT1A, but not 5-HT1B, agonist responsiveness produced by coactivation of the 5-HT2C receptor, further illustrating differences in the capacity of the 5-HT1A and 5-HT1B receptor systems to be regulated by phospholipid signaling cascades.

The 5-HT1A receptor is a target for PKC-mediated phosphorylation, and PKC activation with phorbol esters reduces 5-HT1A receptor signaling (Raymond, 1991; Harrington et al., 1994; Lembo and Albert, 1995; Hensler et al., 1996), although PKC is not involved in mediating the reduction in 5-HT1A agonist efficacy in response to coactivation of P2Y receptors (Evans et al., 2001). However, as shown in Fig. 4, the reduction in responsiveness of the 5-HT1A receptor system produced by pretreatment with ATP was blocked by the protein kinase inhibitor staurosporine and by the selective PKC inhibitor bisindolylmaleimide.

It is well known that PKC activation is a major consequence of activation of PLC. However, more recently it has been shown that stimulation of PKC may also be a consequence of activation of other phospholipases, such as PLA2 and PLD (Jaken, 1996; Exton, 1997), and each of these phospholipases can be activated by P2Y receptors (Briley et al., 1994; Berg et al., 1999; Evans et al., 2001). In the presence of primary alcohols such as ethanol, but not secondary alcohols, the production of the primary product of PLD activation, phosphatidic acid, is blocked (Exton, 1998; Liscovitch et al., 2000). As shown in Fig. 5, the reduction in 5-HT1A agonist responsiveness after ATP pretreatment was blocked in the presence of ethanol, but not in the presence of the secondary alcohol isopropanol.

**Fig. 2.** Effect of ATP pretreatment on agonist efficacy at either 5-HT1A or 5-HT1B receptors. CHO-1A cells were treated with vehicle (distilled H2O) or 1 mM ATP for 5 min at 37°C, washed twice quickly, and further incubated at 37°C for 5, 15, and 30 min as indicated. After this treatment paradigm, inhibition of forskolin-stimulated cAMP accumulation (FscA) (15 min, 37°C) by the 5-HT1A receptor agonist dp-5-CT (10 nM) (A) or 5-HT1B receptor agonist 5-CT (5 nM) (B) in the presence of 10 μM p-MPPF, was measured in the presence and absence of maximal concentrations of the 5-HT2C receptor agonist DOI (1 μM). Data shown are expressed as the percentage of inhibition of FscA by dp-5-CT and 5-CT and are the mean ± S.E.M. of three to five experiments. A, for vehicle-pretreated cells, FscA was 68 ± 8 and 56 ± 7 pmol/mg protein in the absence and presence of DOI, respectively. For ATP-pretreated cells, FscA was 55 ± 10 and 42 ± 10 pmol/mg of protein absence of presence of DOI, respectively. B, for vehicle-pretreated cells, FscA was 78 ± 21 and 87 ± 13 pmol/mg of protein in the presence and absence of DOI, respectively. For ATP-pretreated cells, FscA was 84 ± 22 and 72 ± 13 pmol/mg of protein in the absence and presence of DOI, respectively. *, p < 0.05, ***, p < 0.01 compared with vehicle control and †, p < 0.01 compared with DOI control.

---

**Fig. 3.** Effect of ATP pretreatment on 5-HT2C receptor-mediated cross talk regulation of agonist efficacy at 5-HT1A and 5-HT1B receptors. CHO cells transfected stably with both h-5-HT1A and h-5-HT2C receptors (CHO-2C/1A) were treated with vehicle (distilled H2O) or 1 mM ATP for 5 min (37°C), washed, and further incubated at 37°C for 5 min. After this pretreatment paradigm, the inhibition of forskolin-stimulated cAMP accumulation (FscA) (15 min, 37°C) by the 5-HT1A receptor agonist dp-5-CT (10 nM) (A) or 5-HT1B receptor agonist 5-CT (5 nM) (B) in the presence of 10 μM p-MPPF, was measured in the presence and absence of maximal concentrations of the 5-HT2C receptor agonist DOI (1 μM). Data shown are expressed as the percentage of inhibition of FscA by dp-5-CT and 5-CT and are mean ± S.E.M. of four experiments. FScA after pretreatment and 5-HT1A/1B agonist challenge.
Receptor-mediated activation of PLD has been shown to involve both PKC-dependent and -independent mechanisms (for review, see Exton, 1997). To determine whether PKC activation occurred upstream or downstream to that of PLD, we tested whether the reduction of 5-HT1A receptor system responsiveness by direct activation of PKC with phorbol ester was sensitive to the presence of ethanol. As shown in Fig. 6, ethanol did not block the reduction in 5-HT1A agonist responsiveness produced by activation of PKC with the phorbol ester PDBu.

**Discussion**

Previously, we reported that the responsiveness of the 5-HT1A receptor system can be altered by coactivation of receptors that couple to phospholipid signaling cascades (e.g., 5-HT2C and purinergic P2Y) (Evans et al., 2001). 5-HT1A agonist efficacy is reduced by a cyclooxygenase-dependent metabolite of arachidonic acid that is derived from receptor-mediated activation of PLA2. On the other hand, 5-HT1A agonist efficacy is enhanced by increased [Ca2+], derived from receptor-mediated activation of PLC. The net effect on 5-HT1A receptor system responsiveness is based upon the relative changes in arachidonic acid production versus [Ca2+]i release elicited by phospholipid-coupled receptor activation. Interestingly, although PKC is generally thought to be a consequence of PLC activation and PKC activation with phorbol ester phosphorylates the 5-HT1A receptor and reduces its responsiveness (Raymond, 1991; Harrington et al., 1994; Lembo and Albert, 1995; Hensler et al., 1996), PKC does not seem to play a role in reducing 5-HT1A agonist efficacy in response to coactivation of 5-HT2C or P2Y receptors (Evans et al., 2001).

Herein, we have found that receptor-mediated PKC activation can play a role in regulating the responsiveness of the 5-HT1A receptor system but that the effect is highly dependent upon the timing of receptor activation. Consistent with our previous study, coactivation of P2Y receptors did not alter 5-HT1A agonist responsiveness. However, when the P2Y receptor was activated for 5 min, 5 min before testing the responsiveness of the 5-HT1A receptor system, the 5-HT1A agonist response was reduced. If the time interval between P2Y and 5-HT1A receptor activation was increased to 15 or 30 min, the effect was abolished, suggesting that the effect on 5-HT1A responsiveness is rapidly reversible. The reduction in the 5-HT1A agonist response by P2Y receptor activation is likely mediated by PKC activation because inhibitors of PKC (staurosporine and bisindolylmaleimide) blocked the ATP pretreatment effect.

Currently, 11 isoforms of PKC have been identified that are divided into three classes based on structural properties...
and cofactor requirements. Classical PKC isoforms (i.e., α, βI, βII, and γ) are stimulated by both calcium and 1,2-diacylglycerol or phorbol esters; the novel PKC isoforms (i.e., δ, ε, η, and σ) are activated by diacylglycerol or phorbol esters yet are calcium-independent; and the atypical PKC isoforms (i.e., ζ, λ, and i) are also calcium-independent but are not activated by either diacylglycerol or phorbol esters (Mellor and Parker, 1998). Receptor-mediated diacylglycerol production characteristically occurs in a biphasic manner, such that an initial transient increase in diacylglycerol levels is due to PLC-mediated phosphatidylinositol lipid hydrolysis, whereas a delayed and more sustained increase in diacylglycerol occurs as a result of PLD-mediated phosphatidylcholine lipid hydrolysis and subsequent conversion of phosphatidic acid to diacylglycerol by a phosphatidate phosphohydrolase (Jaken, 1996; Exton, 1997) P2Y receptors have been shown to couple to both PLC (Berg et al., 1996, 1999; Selbie et al., 1997) and PLD (Briley et al., 1994) in CHO cells. To examine the role of PLD in the ATP pretreatment-mediated reduction in 5-HT1A agonist responsiveness, CHO cells were treated with ATP in the presence of ethanol, which blocks the production of phosphatidic acid and the subsequent production of diacylglycerol (Exton, 1998; Liscovitch et al., 2000). Ethanol blocked the ATP pretreatment-mediated reduction in 5-HT1A receptor system responsiveness, suggesting the effect of P2Y receptor activation is mediated by PLD.

Receptor-mediated PLD activation has been shown to involve both PKC-dependent as well as PKC-independent mechanisms involving small molecular weight G proteins such as Rho and ARF (Exton, 1998; Liscovitch et al., 2000). Thus, it is possible that PLD may lie upstream or downstream of PKC. Receptor-mediated activation of PKC could lead to stimulation of PLD with the direct effect on the 5-HT1A receptor system being mediated by consequences of PLD activation (e.g., phosphorytatic acid). Phosphatidic acid targets a number of enzymes, including various protein kinases (including PKC), G protein receptor kinases, and a novel serine/threonine protein kinase, phosphatidic acid-activated protein kinase (McPhail et al., 1999); thus, perhaps a component of the 5-HT1A receptor system could be a direct or indirect target of phosphatidic acid. However, our data suggest that PLD is upstream of PKC because the reduction in 5-HT1A agonist responsiveness elicited by direct activation of PKC with phorbol ester was not blocked by ethanol. Taken together, these data suggest that pretreatment with ATP leads to activation of PLD, which results in a rapidly reversible, PKC-mediated reduction in the responsiveness of the 5-HT1A receptor system. These results are consistent with studies that have shown that activation of PKC with phorbol esters directly phosphorylates the 5-HT1A receptor and reduces 5-HT1A agonist responsiveness (Raymond, 1991; Lembo and Albert, 1995).

Some studies have suggested that activation of diacylglycerol-sensitive PKC isoforms is dependent on the source of diacylglycerol. For example, diacylglycerol derived from PLC activation results in stimulation of calcium-dependent isoforms (e.g., PKCa), whereas PLD-derived diacylglycerol is associated with activation of calcium-independent isoforms (e.g., PKCe) (Ha and Exton, 1993). CHO cells have been reported to express PKC α, δ, ε, τ, and ζ (Megson et al., 2001), which would suggest that the isoform(s) of PKC that mediate the reduction in responsiveness of the 5-HT1A receptor system in response to P2Y receptor activation could be δ or ε.

Although the 5-HT1A and 5-HT1B receptors share a high level of sequence homology and have similar signal transduction systems, it is becoming clear that in addition to similarities, there are significant differences between these receptor systems, especially in their capacity to be regulated. Previously, we found that the responsiveness of both receptor systems is reduced by a cyclooxygenase-dependent metabolite of arachidonic acid derived from activation of PLA2. However the 5-HT1A, but not 5-HT1B, receptor system is regulated by increases in [Ca2+]i. Herein, we found that P2Y-mediated PKC activation, as a result of ATP pretreatment, reduced 5-HT1A, but did not alter 5-HT1B, receptor system responsiveness. This agrees with our previous report that 5-HT1B agonist efficacy is not altered by activation of PKC with phorbol ester and highlights the significant differences between these two receptor systems. Further evidence for differences between the 5-HT1A and 5-HT1B Receptor systems is that ATP pretreatment blocked the effect of 5-HT2C receptor activation on 5-HT1A, but not 5-HT1B, receptor signaling. This specificity for the 5-HT1A receptor is consistent with the notion that the 5-HT1A receptor itself is the target for the ATP pretreatment effect, likely receptor phosphorylation by PKC.

As mentioned above, both 5-HT1A and 5-HT1B receptor system responsiveness is reduced by coactivation of the PLA2 signaling cascade (Berg et al., 1996; Evans et al., 2001). However, the PLA2-arachidonic pathway does not seem to play a role in reducing the responsiveness of the 5-HT1A receptor system in response to ATP pretreatment. Unlike the 5-HT1A receptor system, in response to P2Y receptor activation the 5-HT1B receptor system seems to be exclusively regulated by PLA2, but not by consequences of PLC activation (PKC or Ca2+) (Berg et al., 1996, 1998). With ATP pretreatment conditions that reduced 5-HT1A agonist responses, the responsiveness of the 5-HT1B receptor system was not affected. In fact, none of the pretreatment conditions altered 5-HT1B agonist efficacy. These data suggest that the effect of the cyclooxygenase-dependent metabolite of arachidonic acid on 5-HT1B responsiveness either requires a longer period of P2Y receptor activation (>5 min) or requires coactivation of both receptor systems.

In summary, activation of the P2Y receptor reduces the 5-HT1A receptor system responsiveness in a manner that is dependent upon the temporal pattern of receptor activation. Furthermore, the mechanism for this reduced responsiveness is P2Y-mediated stimulation of PLD, which leads to activation of PKC. It is likely that phosphorylation of the 5-HT1A receptor by PKC, as shown by others (Raymond, 1991), reduces its signaling in response to agonist activation. In contrast, the 5-HT1B receptor system is not regulated in the same time-dependent manner by P2Y receptor activation. Reduced responsiveness of the 5-HT1B receptor system requires coactivation of the P2Y and 5-HT1B receptors. These studies underscore the differences in the 5-HT1A and 5-HT1B receptor systems and emphasize the importance of time as a variable in signal transduction.

References

References


Address correspondence to: Kelly A. Berg, Department of Pharmacology-7764, University of Texas Health Science Center, 7703 Floyd Curl Dr., San Antonio, TX 78229-3900. E-mail: berg@uthscsa.edu