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Central 5-Hydroxytryptamine Receptor-Linked Protein Kinase C Translocation: A Functional Postsynaptic Signal Transduction System

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SUMMARY

The effect of 5-hydroxytryptamine (5-HT) receptor stimulation on protein kinase C (PKC) activity and translocation was assessed in slices or synaptosomes obtained from rat brain. Serotonin (0.5–100 μM) and the specific 5-HT_2 receptor agonist 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) (0.01–10 μM) but not the 5-HT_{1A} or 5-HT_{1B} agonists elicited time- and dose-related translocations in cortical slices. The maximal translocation elicited by 5-HT (10–100 μM, 15 min) or DOI (1 μM, 10 min) was similar to that achievable by the phorbol ester phorbol myristate acetate (PMA) (162 nM). In synaptosomes, short exposures to depolarizing concentrations of K^+ (45–65 mM) resulted in PKC translocation. In addition, PMA but not serotonin induced enzyme translocation in synaptosomes. In slices, serotonin-stimulated PKC translocation was prevented by 5-HT_2 antagonists but not by dopamine or α-adrenergic antagonists. PKC translocation induced by serotonin but not by PMA was inhibited by incubation of slices in a Ca^{2+}-free medium. However, addition of 0.5 mM ethylene glycol bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid to the incubation mixture abolished the effects of both serotonin and PMA. These results indicate that, in cortical slices, serotonin operating via a 5-HT_2 postsynaptic receptor can induce the translocation of PKC from cytosol to membrane. This action of the neurotransmitter appears to be dependent on extracellular Ca^{2+}.

A wide variety of neurotransmitters and hormones have been shown to activate the hydrolysis of PI through interaction with their specific membrane receptors (1). Among these signals, serotonin has repeatedly been shown to interact with 5-HT_2 receptors to initiate the phospholipase C-catalyzed breakdown of phosphoinositol 4,5-bisphosphate (2–8). This subsequently generates two second messengers, DAG and inositol 1,4,5-triphosphate. While inositol 1,4,5-triphosphate mobilizes intracellular Ca^{2+} (9), DAG activates PKC (10, 11).

PKC, a Ca^{2+}- and phospholipid-dependent enzyme, is distributed in both cellular cytoplasmic and membranous compartments. Upon activation, translocation of the enzyme from cytosol to membrane occurs (12, 13). Physiologically, PKC activation can be induced by DAG, which results from receptor-mediated PI hydrolysis (10, 11). In several cultured cell lines, various investigators have shown that receptor-mediated PI hydrolysis leads to a cytosol to membrane redistribution of PKC (14–17). Similarly, translocation of PKC has also been observed in rat pituitary gonadotrophs (18), guinea pig exocrine glands (19), and primary cultures of cerebellar neurons (20) following stimulation with gonadotropin-releasing hormone, carbachol, and glutamate, respectively. In the present communication, we (a) demonstrate that stimulation with serotonin induces a cytosol to membrane redistribution of PKC in various regions of the rat brain and (b) characterize the serotonin receptor subtype that is responsible for this effect in cerebral cortex slices.

Materials and Methods

PKC translocation was examined in brain slices or synaptosomes prepared from cortex, hippocampus, or striatum. Brain tissues were sliced (300 × 300 μm) with a McIlwain tissue chopper, separated, and incubated for 15 min at 37° in oxygenated Krebs-Ringer solution containing various concentrations of agonists. Serotonin receptor-mediated PKC translocation was also examined in a crude synaptosomal preparation [P_2 fraction (21)] obtained from rat cerebral cortex. Synaptosome suspensions were incubated in 0.32 M sucrose/5 mM HEPES (pH 7.5) and treated in the following ways: (a) 15-min buffer, (b) 14.5-min buffer/30-sec 65 mM K^+ stimulation, (c) 15-min buffer containing serotonin, and (d) 15-min buffer containing PMA. Incubation was stopped by the addition of, and sonication (1 min) in, cold 0.32 M sucrose buffered with 20 mM Tris·HCl, pH 7.5, and containing 2 mM

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ABBREVIATIONS: PI, phosphoinositides; PKC, protein kinase C; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; 5-HT, 5-hydroxytryptamine; DOI, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane; PMA, phorbol myristate acetate; 8-OH-DPAT, 8-hydroxy-2-di-*N*-propylaminotetralin; DAG, diacylglycerol; ANOVA, analysis of variance.

EDTA, 0.5 mM EGTA, 50 μg/ml leupeptin, 0.2 mM phenylmethylsulfonyl fluoride, and 0.1% 2-mercaptoethanol. Slices were homogenized in the same buffered sucrose solution. Homogenized slices (22) or sonicated synaptosomes were centrifuged at 800 × *g* for 10 min at 4°. The supernatant was decanted, sonicated (Kontes Micro Cell Disrupter), and centrifuged at 25,000 × *g* for 15 min. The supernatant was applied to DE52 anion exchange columns before measurement of PKC activity (cytosol). The pellet was solubilized on ice for 1 hr in 200 μl of the above buffer containing 1% Nonidet P-40, diluted, and recentrifuged at 25,000 × *g* for 15 min. The supernatant obtained was applied to DE52 columns before PKC activity determination (membrane bound). One-milliliter samples were placed on washed diethylaminomethyl-cellulose columns (Whatman, DE52) and the columns were washed with 5 ml of buffer followed by 0.5 ml of buffer with 0.1 M NaCl. Enzyme was eluted with 1.5 ml of buffer/0.1 M NaCl and used immediately for determination of PKC activity. The final assay mixture (volume of 250 μl) consisted of 20 mM Nacl, 0.1 mM EGTA, 0.4 mM EDTA, 24 mM Tris·HCl, 0.03% 2-mercaptoethanol, 60 μg/ml leupeptin, 0.04 mM phenylmethylsulfonyl fluoride, 0.25 mg/ml histone type III (lysine-rich, Sigma), 1.2 mM $CaCl_2$, 20 μg of phosphatidylserine, 8.1 nM PMA, 10 mM Mg acetate, and 0.03 mM γ [^{32}P]ATP. Assay mixtures were preincubated at 30°. Protein phosphorylation was initiated by the addition of 25 μl of column eluate and performed for 1 min at 30°. Reactions were terminated by transferring 25-μl aliquots of the incubate to a 1 × 2 cm phosphocellulose strip (Whatmat p81) and immersing it in 75 mM phosphoric acid (10 ml/sample). The strips were swirled gently for 2 min, the phosphoric acid was decanted, and the phosphocellulose strips were washed two additional times. After drying in air, the radioactivity was determined by liquid scintillation counting (LKB 1214). Enzyme blanks were assessed for each experiment. Activity showed a greater than 81% sensitivity to phosphatidylserine/PMA. In previous experiments, the reaction was linear for up to 3 min under the identical conditions employed here. Protein was determined by the method of Lowry *et al.* (23) using bovine serum albumin as the standard.

All results are presented as mean ± standard error. The differences between dose- or time-response curves were evaluated using ANOVA analysis followed by Newman-Keuls tests. Statistical significance in data was determined by the two-tailed Student's *t* test.

Results

Effects of serotonin on PKC translocation in cerebral tissues. In cortical slices, 5-HT elicited translocation in a dose- and time-dependent manner (Fig. 1; Table 1). Translocation in response to 5-HT was potentiated by the application of the monoamine oxidase inhibitor pargyline (50 μM) (Fig. 1). Direct PKC activation with the phorbol ester PMA or depolarization with K^+ elicited concentration-related enzyme translocations in cortical slices (Table 2). Serotonin- and PMA-induced PKC redistributions were completely inhibited by 0.5 mM EGTA. However, when tested in Ca^{2+}-free buffer, PMA-induced translocation was unaffected, whereas that elicited by 5-HT or the 5-HT agonist DOI was significantly inhibited (Table 3). In contrast to the results obtained in brain slices, in synaptosomal preparations obtained from the same brain region 5-HT failed to induce PKC translocation. However, direct PKC activation by PMA and K^+-induced depolarization resulted in enzyme translocation (Table 4).

Serotonin-induced PKC translocation was also investigated in hippocampal and striatal slices. In both brain regions, the neurotransmitter was found to elicit a dose-dependent redistribution of the enzyme (Table 5). Enzyme activity was highest in hippocampus > cortex ≥ striatum.

Characterization of cortical serotonin-mediated PKC translocation. In an attempt to characterize the specific 5-HT receptor responsible for activation of PKC translocation, the effects of specific 5-HT agonists were tested in rat cortical slices. Whereas the 5-HT_{1A} and 5-HT_{1B} agonists 8-OH-DPAT and RU24969 were found to be inactive at concentrations of up to 10 μM, the specific 5-HT_2 agonist DOI (24) evoked a concentration-dependent translocation of the enzyme at concentrations of 0.01–1.00 μM (Table 6). The effect of 1 μM DOI was time related. Maximal PKC translocation was achieved after 10 min of incubation with the drug (Fig. 2). Serotonin-induced translocation was prevented by the 5-HT_2 antagonists ketanserin and spiroperidol and was less effectively inhibited by ritanserin, mianserin, and rauwolscine. The response to 5-HT was not affected by the D_2 dopamine receptor antagonist 1-sulpiride (Table 7). These data support the results obtained with the agonists studied and implicate the 5-HT_2 receptors as those mediating the serotonin-induced PKC translocation in cortical slices.

Discussion

The present results demonstrate that, in cortical, hippocampal, and striatal slices, 5-HT induces the translocation of cytosolic PKC to membrane-bound enzyme. In the cortex, this effect of the amine appears to be mediated by postsynaptic 5-

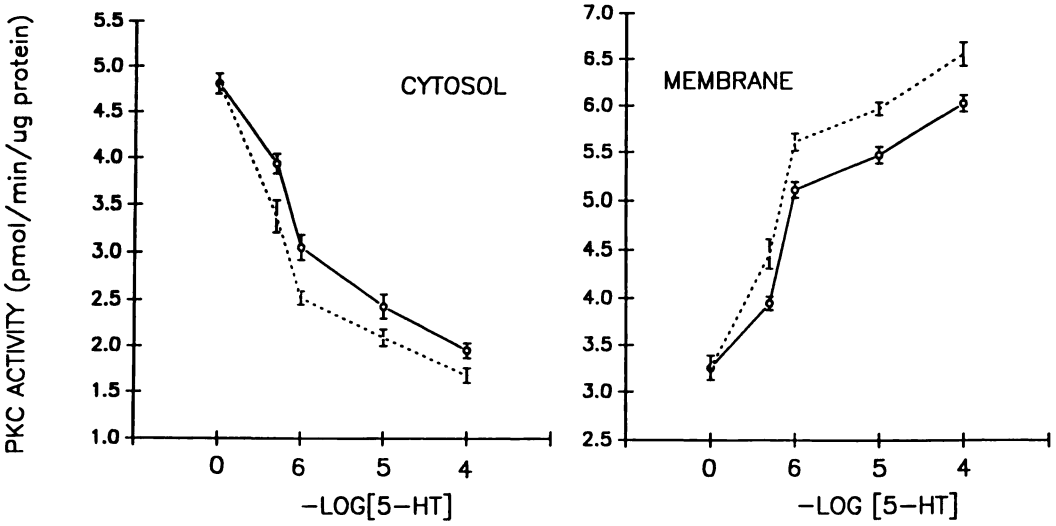

Fig. 1. Effect of serotonin on PKC translocation in cortical brain slices. Tissues were incubated with various concentrations of serotonin in the presence (●——●) or in the absence (○——○) of 50 μM pargyline for 15 min. Cytosolic and membrane-associated PKC activity was determined. Each *point* represents the mean of four separate experiments. *Vertical bars* represents the standard error. The dose-response curves were found to be significant by two-factor ANOVA ($p < 0.01$).

TABLE 1

Time course for serotonin-induced PKC translocation in cortical slices

Cortical brain slices were exposed to 5-HT (10 μM) for various time intervals. Tissues were collected and homogenized and PKC activity was determined in cytosol and membrane fractions. Each value represents the mean ± standard error derived from eight individual experiments. Statistical differences were determined by the two-tailed Student's *t* test.

	PKC activity	
	Cytosol	Membrane-bound
	pmol/min/μg of protein	
Control (Kreb's-Ringer)	4.81 ± 0.09	3.26 ± 0.11
5-HT (5 min)	4.04 ± 0.02[a]	3.56 ± 0.02[b]
5-HT (10 min)	3.43 ± 0.02[c]	4.64 ± 0.02[c]
5-HT (15 min)	2.43 ± 0.11[d]	5.47 ± 0.08[d]
5-HT (20 min)	2.56 ± 0.04[d]	5.59 ± 0.02[d]

[a] $p < 0.01$, [b] $p < 0.05$, [c] $p < 0.005$, [d] $p < 0.001$, when compared with the respective control value.

TABLE 2

PKC translocation in cortical slices in response to various stimuli

Cortical slices were incubated under the conditions indicated in the table. Tissues were collected and homogenized and cytosolic and membrane-associated PKC activities were assessed. Each value represents the results obtained from 4 to 10 individual experiments. Statistical significances were evaluated using two-tailed Student's *t* test.

	PKC activity	
	Cytosol	Membrane-bound
	pmol/min/μg of protein	
Control (Kreb's-Ringer)	5.25 ± 0.10	3.76 ± 0.21
PMA (81 nM, 20 min)	3.57 ± 0.06[a]	5.45 ± 0.09[a]
PMA (162 nM, 20 min)	2.81 ± 0.15[b]	6.13 ± 0.21[b]
K^+ (45 mM, 30 sec)	4.32 ± 0.06[c]	4.81 ± 0.05[c]
K^+ (65 mM, 30 sec)	3.83 ± 0.03[a]	5.47 ± 0.06[a]
5-HT (1 μM, 15 min)	3.38 ± 0.04[b]	5.72 ± 0.20[c]
5-HT (10 μM, 15 min)	2.68 ± 0.04[a]	6.16 ± 0.03[a]

[a] $p < 0.001$, [b] $p < 0.005$, [c] $p < 0.01$, when compared with control (Kreb's Ringer) activity.

TABLE 3

Effect of Ca^{2+} availability on PKC translocation in cortical slices

Cortical slices were incubated for 20 min with 162 nM PMA, 10 μM 5-HT, or 1 μM DOI in the presence or absence of 1.2 mM extracellular Ca^{2+} and with or without EGTA.Tissues were collected and homogenized and cytosolic and membrane-associated PKC activities were assessed. Each value represents the results obtained from 4 to 10 individual experiments. Statistical significances were evaluated using two-tailed Student's *t* test.

	Concentration		PKC activity	
	Ca^{2+}	EGTA	Cytosol	Membrane-bound
	mM		*pmol/min/μg of protein*	
Control (Kreb's-Ringer)	1.2	0	5.11 ± 0.09	3.70 ± 0.09
Ca^{2+}-free Kreb's-Ringer	0	0.5	4.88 ± 0.05	3.79 ± 0.06
PMA	1.2	0	2.74 ± 0.06[a]	5.54 ± 0.09[a]
PMA	0	0	3.06 ± 0.06[a]	5.32 ± 0.11[a]
PMA	0	0.5	4.80 ± 0.14[c]	3.61 ± 0.05[c]
5-HT	1.2	0	2.64 ± 0.06[a]	5.71 ± 0.08[a]
5-HT	0	0	4.23 ± 0.10[b,c]	4.32 ± 0.10[b,c]
5-HT	0	0.5	4.88 ± 0.06[c]	3.93 ± 0.06[c]
DOI	1.2	0	2.69 ± 0.09[a]	5.89 ± 0.12[a]
DOI	0	0	3.98 ± 0.05[b,c]	4.41 ± 0.04[b,c]

[a] $p < 0.001$, [b] $p < 0.01$, when compared with PKC activity in the control fraction. [c] $p < 0.001$ when compared with the respective response induced in the presence of 1.2 mM of Ca^{2+}.

HT receptors, as suggested by the fact that 5-HT was unable to induce redistribution of PKC in synaptosomal preparations obtained from the same brain area and by the 5-HT response observed in cortical slices taken from animals with serotonergic

TABLE 4

Effect of various stimuli on PKC translocation in cortical synaptosomes

Synaptosomes were prepared from rat cortex and exposed to various agents as indicated. Reaction was terminated by diluting with 0.5 mM EGTA-containing Ca^{2+}-free buffer and immediately sonicated. Cytosolic and membrane-bound fractions were obtained by centrifugation and PKC activities were assessed. Each value represents the mean ± standard error of four to six individual experiments. Statistical differences were evaluated by two-tailed Student's *t* test.

	PKC activity	
	Cytosol	Membrane-bound
	pmol/min/μg of protein	
Control (Kreb's-Ringer)	3.60 ± 0.05	2.69 ± 0.03
K^+ (65 mM, 30 sec)	1.82 ± 0.07[a]	4.45 ± 0.13[b]
PMA (162 nM, 15 min)	2.29 ± 0.07[a]	4.16 ± 0.21[b]
5-HT (10^{-5} M, 15 min)	3.54 ± 0.14	2.82 ± 0.08
5-HT (10^{-4} M, 15 min)	3.47 ± 0.05	2.35 ± 0.05

[a] $p < 0.005$ when compared with control value.

TABLE 5

Serotonin-induced PKC translocation in various brain areas

Brain slices were exposed to various concentrations of serotonin. Tissues were collected and homogenized and PKC activities were determined in cytosol and membrane fractions. Each value represents the mean ± standard error derived from 4–15 individual experiments. The concentration-response curves were subjected to one-factor ANOVA ($p < 0.01$ for cortex and hippocampus, $p < 0.05$ for striatum) followed by Neuman-Keuls test.

	PKC activity	
	Cytosol	Membrane-bound
	pmol/min/μg of protein	
Cerebral cortex		
Control	4.45 ± 0.07	3.19 ± 0.08
5-HT (10^{-6} M)	3.05 ± 0.11[a]	5.10 ± 0.06[a]
5-HT (10^{-5} M)	2.43 ± 0.11[b]	5.47 ± 0.08[b]
5-HT (10^{-4} M)	1.75 ± 0.06[b]	6.03 ± 0.07[b]
Hippocampus		
Control	6.26 ± 0.02	3.78 ± 0.06
5-HT (10^{-6} M)	3.94 ± 0.09[a]	6.47 ± 0.19[a]
5-HT (10^{-5} M)	3.22 ± 0.07[b]	7.14 ± 0.09[b]
5-HT (10^{-4} M)	2.68 ± 0.03[b]	8.02 ± 0.07[b]
Striatum		
Control	4.14 ± 0.02	3.12 ± 0.06
5-HT (10^{-6} M)	3.24 ± 0.02[c]	4.38 ± 0.05[c]
5-HT (10^{-5} M)	2.78 ± 0.09[c]	5.18 ± 0.20[c]
5-HT (10^{-4} M)	2.51 ± 0.03[b]	5.60 ± 0.16[b]

[a] $p < 0.005$, [b] $p < 0.001$, [c] $p < 0.01$, when compared with the respective control value.

neuronal lesions induced by the neurotoxin 5,7-dihydroxytryptamine.[1] PKC translocation in synaptosomal preparations was, however, induced by direct enzyme stimulation with phorbol ester or by K^+-induced depolarization. The data, therefore, suggest that in brain slices PKC translocation may be initiated via both presynaptic (depolarization) and postsynaptic (neurotransmitter receptor) mechanisms. The findings of PKC translocation at these sites are consistent with physiological roles for PKC-mediated phosphorylation in neurotransmitter release (25, 26) and in postsynaptic receptor-mediated functions (27).

The 5-HT-stimulated PKC translocation in cortical slices appears to be mediated by a serotonin receptor with pharmacological properties compatible with those of 5-HT_2 receptors. This is supported by the findings that (a) the selective 5-HT_2 agonist DOI but not the 5-HT_{1A} and 5-HT_{1B} agonists 8-OH-DPAT and RU24969 elicited PKC translocation and (b) 5-HT-

[1] Unpublished data.

TABLE 6

Effect of specific 5-HT agonists on PKC translocation in cortical slices

Cortical brain slices were exposed to various concentrations of serotonin agonists. Tissues were collected and homogenized and PKC activities were determined in cytosol and membrane fractions. Each value represents the mean ± standard error derived from 10–24 individual experiments. DOI dose-response curves were found to be significant in one-factor ANOVA followed by Neuman-Keuls test.

	PKC activity	
	Cytosol	Membrane-bound
	pmol/min/μg of protein	
Control	4.81 ± 0.09	3.26 ± 0.11
5-HT_{1A}		
8-OH-DPAT (1 μM)	4.55 ± 0.05	3.41 ± 0.09
8-OH-DPAT (10 μM)	4.39 ± 0.14	3.42 ± 0.08
5-HT_{1B}		
RU 24969 (1 μM)	4.70 ± 0.09	3.42 ± 0.08
RU 24969 (10 μM)	4.56 ± 0.08	3.35 ± 0.05
5-HT_2		
DOI (0.01 μM)	3.89 ± 0.05[a]	4.36 ± 0.07[a]
DOI (0.10 μM)	2.98 ± 0.06[b]	5.57 ± 0.07[b]
DOI (1.00 μM)	1.97 ± 0.08[b]	6.29 ± 0.09[b]
DOI (10.0 μM)	1.86 ± 0.12[b]	6.23 ± 0.09[b]

[a] $p < 0.005$, [b] $p < 0.001$, when compared with the respective control value.

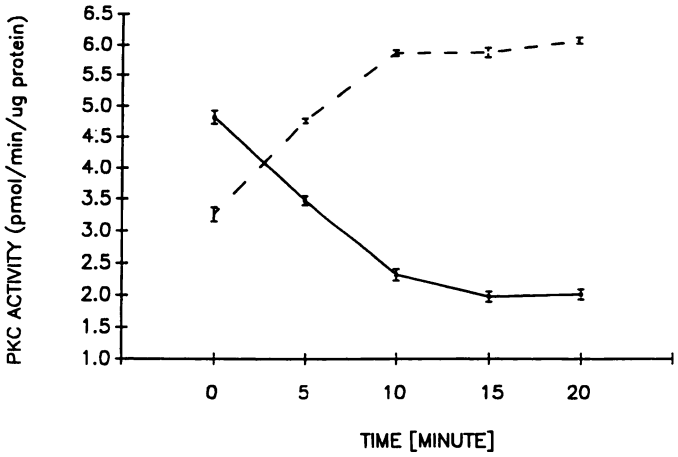

Fig. 2. Effect of DOI on PKC translocation in cortical slices. Cortical brain slices were exposed for various time intervals to 1 μM DOI. Tissues were collected and homogenized and PKC activity was determined in cytosolic (●——●) and particulate (●– – –●) fractions. Each *point* represents the mean of four separate experiments. *Vertical bars* indicate the standard error. The time effect was found to be significant by one factor ANOVA ($p < 0.01$).

induced enzyme redistribution was prevented by the selective 5-HT_2 antagonists ketanserin and spiroperidol and only weakly inhibited by the less selective serotonin antagonists (28). The results are consistent with previous observations indicating that activation of 5-HT_2 receptor-linked phospholipase C results in PI hydrolysis (3, 4). They further suggest that the 5-HT-induced cortical PKC translocation observed in the present investigation is a consequence of the intracellular elevation in diacylglycerol resulting from receptor-mediated PI hydrolysis.

Our results indicated that intracellular Ca^{2+} is required for the translocation process per se, because translocation induced by PMA or 5-HT was completely abolished following incubation of tissues with EGTA. This observation is in agreement with the notion that Ca^{2+} increases the affinity of PKC for cerebral membranes (10). The data also suggest that 5-HT- (but not PMA-) evoked PKC translocation is mediated by phospholipase C activation, a reaction that has been shown to

TABLE 7

Effect of antagonists on serotonin-induced PKC translocation in cortical slices

Cortical brain slices were exposed to 10 μM serotonin or the combination of serotonin and antagonists (10 μM). Tissues were collected and homogenized and PKC activities were determined in cytosol and membrane fractions. Each value represents the mean ± standard error derived from 8–24 individual experiments. Statistical differences were determined by the two-tailed Student's *t* test.

	PKC activity	
	Cytosol	Membrane-bound
	pmol/min/μg of protein	
Control	4.81 ± 0.09	3.26 ± 0.11
5-HT	2.43 ± 0.11[a]	5.47 ± 0.08[b]
5-HT + ketanserin	4.31 ± 0.06[c]	3.32 ± 0.04[c]
5-HT + spiroperidol	4.26 ± 0.07[c]	2.94 ± 0.14[c]
5-HT + mianserin	2.87 ± 0.05[d]	5.00 ± 0.04[d]
5-HT + ritanserin	2.89 ± 0.13[d]	5.06 ± 0.10[d]
5-HT + sulpiride	2.44 ± 0.05[a]	5.27 ± 0.06[b]
5-HT + rauwolscine	2.99 ± 0.04[d]	4.87 ± 0.12[d]

[a] $p < 0.001$, [b] $p < 0.005$, when compared with the respective control level.
[c] $p < 0.001$, [d] $p < 0.01$, when compared with the respective serotonin-induced response.

depend on extracellular Ca^{2+} in cerebral cortical slices (29). Alternatively, the present results might suggest that 5-HT-elicited PKC translocation is mediated by Ca^{2+} influx, as reported for mammalian and *Aplysia* neurons (30, 31) or indirectly through a second neuron.

A large body of evidence strongly suggests that PKC is present in high concentrations in the brain (32, 33), where it plays a pivotal role in neurotransmitter- or hormone-mediated signal transduction. PKC-mediated neuronal protein phosphorylation mediates a wide variety of neurophysiological events such as membrane conductance, ion channel conductivity, and axonal transport (34). Serotonergic neurons may regulate their specific target neurons through 5-HT_2 receptor-mediated PI hydrolysis and/or PKC activation. Characterization of these 5-HT_2 receptor-linked signal transduction systems may, therefore, advance our understanding of serotonergic neurotransmission.

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