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Short communication

Desensitization of 5-hydroxytryptamine-facilitated dopamine release in vivo

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Abstract

Desensitization to serotonin (5-hydroxytryptamine, 5-HT)-facilitated dopamine release in vivo develops following either continuous or repeated pulses of 5-HT. For instance, an initial 20 min pulse of 5-HT (10 μM) produced a 715 $\pm$ 150% increase in basal dopamine, an effect which steadily declined over 5 subsequent fractions to 222 $\pm$ 80%. Also, secondary pulses (s2) of 5-HT administered 40 min following the primary pulse (s1), elicited a 327 $\pm$ 16% increase in dopamine levels, significantly decreased from the primary s1 pulse. Augmentation of either protein kinase A or protein kinase C systems attenuated 5-HT-facilitated dopamine release, suggesting a role for protein kinases in regulating the desensitization process.

Keywords: Dopamine; 5-HT (5-hydroxytryptamine, serotonin); Desensitization; Striatum; Protein kinase A; Protein kinase C; (Rat)

1. Introduction

Several recent studies investigating the functional responses to pharmacological activation of central 5-HT_1, 5-HT_2, 5-HT_3, and 5-HT_4 receptors suggest these responses are susceptible to protein kinase-mediated receptor desensitization (Ansanay et al., 1992; Boddeke et al., 1993; Kagaya et al., 1990; Leysen et al., 1989; Rahman and Neuman, 1993; Raymond, 1991; Yakel and Jackson, 1988). For instance, 5-HT-induced depolarization of neostriatal neurons is mediated at least partially via a rapidly inactivating, fast inward sodium current dependent on 5-HT_3 receptor activation. The 5-HT_3 receptor-ion channel complex is subject to protein kinase A-mediated desensitization, a process which can be mimicked and accelerated by elevations in intracellular cAMP levels (Yakel and Jackson, 1988). Furthermore, stimulation of 5-HT_{1a}, 5-HT_{1c}, and 5-HT_2 receptors expressed in human and rodent cell lines and platelets is sensitive to protein kinase C-mediated desensitization (Boddeke et al., 1993; Kagaya et al., 1990; Raymond, 1991). Also, like β-adrenoceptors, the 5-HT_4 receptor is susceptible to both β-adrenoceptor kinase-mediated desensitization and receptor sequestration (Ansanay et al., 1992).

Although the facilitatory effect of 5-HT on striatal dopamine release has been established (Benloucif et al., 1993; Benloucif and Galloway, 1991; Bonhomme et al., 1995; West and Galloway, in press; Yadid et al., 1994), the effects of multiple acute 5-HT pulses and long-term 5-HT administration on dynamic dopaminergic function has not been determined. In order to study further the nature of this 5-HT-dopamine interaction and its potential susceptibility to desensitization, we examined the effects of multiple and continuous pulses of 5-HT on striatal dopamine release in vivo. The potential involvement of protein kinase A and protein kinase C-mediated mechanisms in regulating the 5-HT-facilitated dopamine release and desensitization processes was investigated using the adenylyl cyclase activator, forskolin, and the protein kinase C activator, phorbol 12,13 myristoyl-acetate (PMA). Determination of post-receptor regulatory pathways utilized in 5-HT-induced dopamine release may lead to the development of pharmaceuticals aimed at increasing the long-term efficacy of 5-HT as a dopamine releasing agent.

2. Materials and methods

2.1. General

Microdialysis was carried out as previously described (West and Galloway, in press). Briefly, in each subject,

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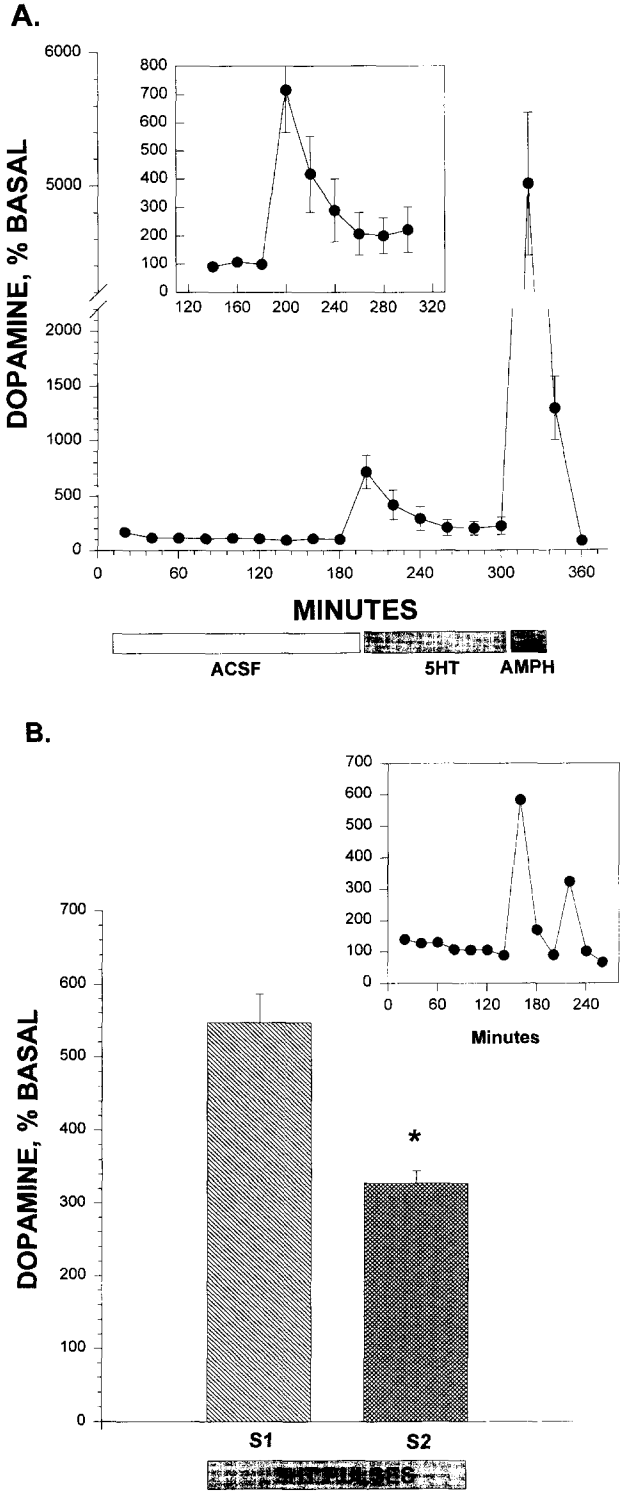
two dialysis probes of the concentric design were implanted bilaterally in the anterior medial striatum of chloral hydrate-anesthetized (400 mg/kg i.p.) male rats. Probes were perfused at a rate of 2 μl per minute with artificial cerebral spinal fluid (ACSF) containing (mM) 145 Na^+, 2.7 K^+, 1.0 Mg^{2+}, and 1.2 Ca^{2+}, maintained at pH = 7.4 with 2 mM sodium-phosphate buffer. Samples were collected every 20 min (represents one fraction) into 10 μl of high pressure liquid chromatography (HPLC) mobile phase to minimize degradation of neurotransmitters and were immediately analyzed by HPLC with electrochemical detection (Benloucif and Galloway, 1991).



2.2. Drug administration

Drugs were administered following stabilization of dopamine levels in the dialysate. Three consecutive 20 min fractions varying less than 20% in dopamine concentration constituted a stable baseline, which occurred approximately 2–3 h following probe implantation. Subjects received 0.4 nmol 5-HT (10 μM in the dialysate) perfused through the probe per 20 min fraction. Other animals received either forskolin (100 μM) or PMA (1 μM) perfused for 100 min following baseline fractions. Additionally, following pretreatment either forskolin (100 μM) or PMA (1 μM) was coperfused with 5-HT (10 μM) during one 20 min fraction. 5-HT and *d*-amphetamine sulphate were dissolved in ACSF, while forskolin and PMA were dissolved in absolute ethanol and diluted to 1%. Data from previous experiments have shown that ethanol at this concentration has no effect on basal dopamine release (data not shown).

2.3. Data analysis

Student's *t*-test, one-way analysis of variance with repeated measures (ANOVA-RM) with Dunnet's multiple comparisons test, or one-way analysis of variance (ANOVA) with Bonferroni *t*-test (as indicated) were used to assess the statistical significance of drug induced changes in extracellular dopamine levels.

Fig. 1. Panel A: Desensitization of 5-HT-facilitated dopamine release: continuous perfusion. Continuous local perfusion of 5-HT (10 μM) results in an initial 715 ± 149% increase over basal extracellular dopamine levels ($P < 0.01$). In subsequent fractions, the facilitatory effect of 5-HT on dopamine release significantly diminished during continuous administration ($P < 0.05$, as compared to the response to the initial 5-HT pulse using ANOVA-RM with Dunnett's multiple comparisons test). Intrastriatal infusion of *d*-amphetamine sulphate (10 μM) immediately following 5-HT administration significantly increased dopamine levels to 5012 ± 535% above basal levels. Inset: Enlargement of the effect of continuous perfusion of 5-HT on extracellular dopamine. Symbols represent the mean ± S.E.M. for $n = 4–5$ experiments. Basal extracellular dopamine levels were 0.71 ± 0.08 fmol/μl. Panel B: Desensitization of 5-HT-facilitated dopamine release: repeated pulses. Local perfusion of 5-HT (10 μM) (s1) increased extracellular dopamine levels 547 ± 13% over baseline ($P < 0.001$). Following a 40 min recovery period and a return to basal dopamine levels, a second pulse (s2) of 5-HT increased extracellular dopamine levels to 327 ± 16%. Comparison of the 5-HT s1 and s2 pulses using a paired *t*-test revealed a significant decrease in 5-HT facilitation of dopamine release following the initial pulse (* $P < 0.01$). Bars represent the mean ± S.E.M. for $n = 5$ experiments. Basal dopamine levels were 0.71 ± 0.08 fmol/μl. The inset demonstrates a representative trace of basal and 5-HT-stimulated (s1, s2) dopamine release for a typical experiment.

3. Results

3.1. Desensitization of 5-HT-facilitated dopamine release

To further characterize 5-HT-facilitated dopamine release and determine the response to prolonged 5-HT infusion, multiple 5-HT pulses were administered. In Fig. 1A, continuous perfusion of 5-HT (10 μM) resulted in an initial 715 ± 150% (first fraction) increase in extracellular dopamine levels ($P < 0.01$). ANOVA-RM and Dunnett's multiple comparisons test on six consecutive fractions receiving 5-HT revealed that a significant diminution in 5-HT-induced dopamine release occurred in response to continuous 5-HT administration ($P < 0.05$). This desensitization of the 5-HT effect was not due to a lack of transmitter availability as amphetamine (10 μM) perfusion following continuous 5-HT induced a robust 5000-fold elevation over basal dopamine concentrations.

In order to eliminate the potential influence of dopamine autoreceptor activation during continuous 5-HT perfusion and periods of elevated dopamine release, the effects of separate repeated exposures to 5-HT were investigated. As shown in Fig. 1B, the primary pulse of 5-HT produced a 5- to 6-fold increase in extracellular dopamine levels. When administered 40 min after the initial 5-HT pulse, the secondary pulse (s2) of 5-HT elicited only a 3-fold increase in extracellular dopamine levels. This diminution of dopamine releasing efficacy observed for the secondary 5-HT pulse was significantly less than that of the primary 5-HT pulse ($P < 0.01$, as determined by paired t-test). The apparent tachyphylaxis developed to a single 20 min pulse (s2) of 5-HT delivered following a 40 min recovery period, during which time dopamine levels had returned to baseline (pre-serotonin) levels.

Table 1
The effects of 100 min of pretreatment and coperfusion of PMA and forskolin on 5-HT-facilitated dopamine release

Pretreatment	Dopamine, fmol/μl (basal levels)	Drug perfusion (% basal control)	5-HT perfusion (% drug control)
None	2.26 ± 0.16	–	702 ± 89 [a]
PMA (1 μM)	1.02 ± 0.04 [d]	104 ± 1.0	461 ± 55 [b]
Forskolin (100 μM)	1.92 ± 0.18	216 ± 6.0 [a]	258 ± 55 [c]

Data represent the mean ± S.E.M. for $n = 6–10$ experiments. [a] Significant increase in extracellular dopamine over basal levels, $P < 0.01$ as determined by unpaired t-test. [b] Significant attenuation in 5-HT-mediated dopamine release, $P < 0.05$ as compared to 5-HT control group using ANOVA with Bonferroni t-test. [c] Significant decrease in 5-HT-facilitated dopamine release, $P < 0.01$ as compared to 5-HT control group using ANOVA with Bonferroni t-test. [d] Basal dopamine levels for this group were significantly lower than those of the 5-HT control and forskolin groups as demonstrated by ANOVA with Bonferroni t-test. However, basal dopamine levels for the PMA group were within the normal range commonly observed in our laboratory (1–3 fmol/μl).

3.2. Mechanism of desensitization of 5-HT-facilitated dopamine release

In order to examine the role of protein kinase C and protein kinase A in 5-HT receptor desensitization, the protein kinase C activator PMA (1 μM) and the adenylyl cyclase activator forskolin (100 μM) were delivered prior to and during the 5-HT pulse (Table 1). 100 min of intrastriatal perfusion with PMA (1 μM) did not affect basal extracellular dopamine levels (Table 1). Similar treatment with forskolin (100 μM) increased extracellular dopamine levels 116% over basal levels (Table 1). In control subjects, the 5-HT pulse resulted in a 702% increase in extracellular dopamine levels. Pretreatment and coperfusion with either PMA or forskolin attenuated the magnitude of the 5-HT response to 461 ± 55% and 258 ± 55% respectively.

4. Discussion

The results from this study reveal that 5-HT-facilitated dopamine release undergoes desensitization following either continuous (2 h) perfusion or multiple 5-HT pulses. This phenomenon of desensitization, or the diminished functional response in the continued presence of agonist is widespread and has been reported to occur with prolonged exposure to many neurotransmitters (Dillon-Carter and Chuang, 1989). The classic studies of adrenoceptor desensitization in vitro by Lefkowitz and colleagues (for review see Hausdorff et al., 1990), suggest that acute desensitization occurs primarily via a protein kinase-dependent uncoupling of the receptor from its respective G-protein. This assumption was tested indirectly in our study using protein kinase activators to bolster potential kinase-dependent regulation of 5-HT receptor function. Additionally, the state of intraneuronal dopamine stores and the role of dopamine autoregulatory mechanisms were assessed as potential non-receptor related desensitization mechanisms.

The possibility that continuous exposure to 5-HT depletes terminal dopamine pools was tested with a post-desensitization administered pulse of amphetamine. The tachyphylaxic response did not result from an unavailability of releasable cytosolic dopamine, as a robust response to amphetamine was elicited following desensitization to continuous 5-HT treatment. Although the potential depletion of dopamine from vesicular stores by prolonged 5-HT administration was not directly tested, it is probable that the amphetamine-induced release of dopamine represented both cytosolic and vesicular dopamine pools as amphetamine has been shown to redistribute dopamine from synaptic vesicles to the cytosol (Sulzer et al., 1995). Also, the kinetics of vesicular membrane recycling suggest that vesicle availability is not a factor in the desensitization process.

It is unlikely that dopaminergic autoregulatory mecha-

nisms account for the decreased responsiveness to 5-HT (s2) administered 40 min after the initial 5-HT pulse (s1) as extracellular dopamine levels returned to basal levels in between 5-HT pulses (Fig. 1B inset). Thus, the desensitization state was observed after a recovery period during which extracellular dopamine levels had normalized, suggesting any inhibitory tone presented by elevated synaptic dopamine affecting autoreceptors or negative feedback loops would be eliminated prior to the second 5-HT pulse. The role of dopamine autoregulatory mechanisms during desensitization to continuous 5-HT perfusion (Fig. 1A) remains to be assessed.

As multiple studies in vitro demonstrate that acute desensitization of several 5-HT receptor subtypes is mediated via protein kinase activation (Boddeke et al., 1993; Dillon-Carter and Chuang, 1989; Harrington et al., 1994; Kagaya et al., 1990; Rahman and Neuman, 1993; Raymond, 1991; Yakel and Jackson, 1988), we tested the potential role of protein kinase A and protein kinase C in the desensitization to 5-HT-facilitated dopamine release in vivo. The potential involvement of protein kinase A and protein kinase C activation in the desensitization process was tested indirectly via the pretreatment and coperfusion of either forskolin or PMA, prior to and during the 5-HT pulse (Table 1). Presumably, if the desensitization mechanism involved a receptor-mediated stimulation of adenylyl cyclase and consequent cAMP-dependent protein kinase A activation, then augmentation of this key enzyme should facilitate the desensitization process. Likewise, if a receptor-mediated activation of protein kinase C regulates the desensitization process, then stimulation of this kinase should mimic the tachyphylaxis observed to the 5-HT s2 pulse. Pretreatment and coperfusion with forskolin attenuated 5-HT-facilitated dopamine release similar to the magnitude of desensitization exhibited during the s2 5-HT pulse shown on Fig. 1B. PMA pretreatment decreased 5-HT-facilitated dopamine release to a lesser extent. These results suggest that both protein kinase A and protein kinase C may play a role in desensitization of 5-HT-facilitated dopamine release.

Although the current study did not focus on the specific 5-HT receptor subtype(s) involved in desensitization to 5-HT-facilitated dopamine release in vivo, recent studies using single pulses of 5-HT suggest multiple 5-HT receptor subtypes may be involved (Benloucif et al., 1993; Bonhomme et al., 1995). 5-HT-facilitated dopamine release in the striatum can be at least partially antagonized by coperfusion with pindolol, ICS 205930, MDL 7222 (Benloucif et al., 1993), and DAU 6285 (Bonhomme et al., 1995) in a dose-dependent manner. Although a consensus as to the 5-HT receptor(s) involved in 5-HT-facilitated dopamine release has not been reached, the results cited above suggest that 5-HT_1, 5-HT_3, and 5-HT_4 receptors may be involved in mediating the desensitization process.

In summary, the current study demonstrates that 5-HT facilitation of striatal dopamine release undergoes a desensitization process following continuous or repeated exposure to 5-HT. Augmentation of either the protein kinase C or cAMP/protein kinase A second messenger cascades alters 5-HT-facilitated dopamine release. Thus, protein kinase-dependent modulation of receptor function may represent an intracellular mechanism for the regulation of striatal dopaminergic neurotransmission.

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