Published in final edited form as:

Brain Res. 2007 November 28; 1182: 82–89.

Dopamine Release in Prefrontal Cortex in Response to β -Amyloid Activation of $\alpha 7^*$ Nicotinic Receptors

Jianlin Wu^{*}, Ghous M. Khan, and Robert A. Nichols[‡]

Department of Pharmacology & Physiology, Drexel University College of Medicine, Philadelphia, PA 19102

Abstract

The levels of soluble beta amyloid ($A\beta$) are correlated with symptom severity in Alzheimer's disease. Soluble $A\beta$ has been shown to disrupt synaptic function and it has been proposed that accumulation of soluble $A\beta$ triggers synapse loss over the course of the disease. Numerous studies indicate that soluble $A\beta$ has multiple targets, one of which appears to be the nicotinic acetylcholine receptor, particularly for $A\beta$ concentrations of pM-nM. Moreover, pM-nM soluble $A\beta$ was found to increase presynaptic Ca^{2+} levels, suggesting that it may have an impact on neurotransmitter release. In the present study, soluble $A\beta$ was perfused into mouse prefrontal cortex and the effect on the release of dopamine outflow via microdialysis was assessed. In the presence of tetrodotoxin, $A\beta_{1-42}$ at 100nM evoked the release of dopamine to ~170% of basal levels. The $A\beta_{1-42}$ -evoked dopamine release was sensitive to antagonists of $\alpha 7$ nicotinic receptors and was absent in mice harboring a null mutation for the $\alpha 7$ nicotinic subunit, but was intact in mice harboring a null mutation for the $\beta 2$ nicotinic subunit. The control peptide $A\beta_{40-1}$ was without effect. In contrast, $A\beta_{1-42}$ at 1-10pM caused a profound but slowly developing decrease in dopamine outflow. These results suggest that $A\beta$ alters dopamine release in mouse prefrontal cortex, perhaps involving distinct targets as it accumulates during Alzheimer's disease and leading to disruption of synaptic signaling.

Section: Disease-Related Neuroscience

Keywords

Prefrontal Cortex; Beta Amyloid; Dopamine Release; In Vivo Microdialysis

1. Introduction

One of the major pathological entities in Alzheimer's disease (AD) is beta amyloid ($A\beta$). $A\beta$ refers to a collection of peptides of 38-43 amino acids in length, which are derived from the amyloid precursor protein (APP) by sequential proteolytic cleavage (reviewed in Walsh and Selkoe, 2007). The predominant forms of $A\beta$ found in AD are $A\beta_{1-40}$ and $A\beta_{1-42}$, with their levels progressively increasing over the course of the disease (Ingelsson et al., 2004). Though $A\beta$ notably accumulates in plaques, a significant portion exists in soluble forms, mainly as oligomers (or ADDLs; Lambert et al., 1998), and it has been proposed that increased levels of soluble $A\beta$ oligomers in the early stages of AD cause synaptic dysfunction (Hardy and Selkoe,

^{*}Present address: Lutheran Medical Center, Brooklyn, NY 11220

[‡]Correspondence: Dr. R.A. Nichols, Ph.D., Department of Pharmacology & Physiology, Drexel University College of Medicine, 245 N. 15th Street, Philadelphia, PA 19102, USA, E-Mail: robert.nichols@drexel.edu, Tel: (215)762-2383; Fax: (215)762-2299

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2002; Selkoe and Schenk, 2003) and, later, synapse loss (Lue et al., 1999). The A β ₁₋₄₂ form, though less abundant than the A β ₁₋₄₀ form, has the highest propensity to form toxic oligomers (Walsh and Selkoe, 2007) and correlates with disease onset (Kumar-Singh et al., 2006). In addition, aggregated and fibrillar forms of A β also accumulate and hence different forms of A β may have different effects in the brain, which, together with neurofibrillary pathology, likely includes synaptic dysfunction, synapse loss, oxidative damage, inflammation and neuron loss (Selkoe, 2002; Mattson, 2004).

The accumulation of A β in AD is selective, with particularly high concentrations found in frontal cortex, entorhinal cortex, hippocampus, amygdala and parietal association cortex (Braak and Braak, 1991). Moreover, the accumulation of A β is largely linked to its release from synaptic nerve endings, as evidenced by its loss from terminal innervation sites of lesioned pathways in APP transgenic mice (Lazarov et al., 2002; Sheng et al., 2002). In addition, soluble A β oligomers, particularly A β ₁₋₄₂, has been shown to disrupt synaptic plasticity, specifically LTP in the hippocampus (Walsh et al., 2005). On the other hand, it appears that oligomerization of A β starts intraneuronally (Oddo et al., 2006). The extent to which A β increases or decreases synaptic activity, and its site(s) of action, remains to be fully elucidated.

Previous work has demonstrated a potent action of soluble A β on nicotinic acetylcholine receptors (nAChRs), including both antagonist (Pettit et al., 2001; Liu et al., 2001; Grassi et al., 2003; Wu et al., 2004) and agonist (Dineley et al., 2001; Dougherty et al., 2003; Fu et al., 2003) effects. We have shown that pM-nM A β ₁₋₄₂ induces increased [Ca²⁺]_i in isolated presynaptic terminals from rat cortex and hippocampus in a manner susceptible to partial antagonism by classical nAChR antagonists, such as α -bungarotoxin and dihydro- β -erythroidine. Moreover, prior activation of presynaptic nAChRs attenuated subsequent responses to A β ₁₋₄₂. However, there remains some reservation regarding the actual target for soluble A β , particularly the role of nAChRs, and whether activation of presynaptic nAChRs leads to altered synaptic transmission. Here, we employed preparations from mice harboring null mutations for either the $\alpha 7$ subunit or the $\beta 2$ subunit of the two major nAChR subtypes present in brain, namely the α -bungarotoxin-sensitive and high affinity subtypes, respectively (Role and Berg, 1996; Zoli et al., 1998) to examine the effect of A β on the release of dopamine in the prefrontal cortex of the intact brain. Dopamine (DA) is a prominent player in the functioning of the prefrontal cortex (Arnsten and Li, 2005) and alterations in its release by A β could lead to altered prefrontal cortical function.

2. Results

2.1 Nicotine and β -amyloid evoke the release of dopamine in mouse prefrontal cortex

The release of dopamine in prefrontal cortex was measured in freely moving mice via *in vivo* microdialysis (Fig. S1) in the presence of TTX in order to isolate presynaptic regulation from cellular effects. Nicotine (1 μ M) in the presence of TTX evoked an increase in DA outflow ($\sim$ 300% over basal) and this increase was blocked by prior treatment with a nAChR antagonist (Fig. 2A). These results are generally consistent with previous reports of nicotine-evoked DA release in rodent prefrontal cortex slices (Rao et al., 2003; Cao et al., 2005). Perfusion with soluble 100nM of A β ₁₋₄₂ (Fig. 1) also evoked the release of DA ($\sim$ 170% over basal; $p < 0.05$) in a manner sensitive to nAChR antagonists (Fig. 2B). (Perfusion of A β using microdialysis has been previously characterized (Parks et al., 2001; Trabace et al., 2007).) Perfusion with 100nM of the soluble core amyloid fragment A β ₁₂₋₂₈ (Wang et al., 2000a; Dougherty et al., 2003) also evoked the release of DA (Fig. 2C), but the response was rather variable. Curiously, perfusion with 1-10pM A β ₁₋₄₂ did not evoke an increase in DA outflow, but, rather, caused a slowly developing, profound decrease in DA outflow (Fig. 2D), down to just detectable levels (fg/ μ l DA in perfusate). (With shorter time of perfusion of 100pM or 100nM A β , the DA outflow was decreased at later time points after initially evoking an increase (see Fig. 3); longer

times of perfusion with A β likely did not allow the peptide concentration to fall to pM over the course of the recording.) The decrease in DA release was not significantly affected by nAChR antagonist (not shown). Perfusion with the control peptide, "A β ₄₀₋₁", was without effect (Fig. 2E).

2.2 β -amyloid evoked release of dopamine is mediated by $\alpha 7^*$ nAChRs

The pharmacological assessment of the A β -evoked DA release in prefrontal cortex indicated involvement of $\alpha 7^*$ nAChRs, based on inhibition by α -bungarotoxin (Fig. 2B). To ascertain definitely the role of particular nAChR subtypes, the A β -evoked DA release was examined in mice harboring null mutations for either the $\alpha 7$ subunit or $\beta 2$ subunit of nAChRs. The DA outflow evoked by 100nM A β ₁₋₄₂ was absent in $\alpha 7$ null-mutant mice, whereas the inhibition of DA release remained (Fig. 3A). By contrast, DA release evoked by 100nM A β ₁₋₄₂ was intact in $\beta 2$ null-mutant mice (Fig. 3B). Prior treatment with nicotine attenuated the subsequent A β -evoked DA outflow, but did not affect the slowly developing decrease in DA release (Fig. 3C). These results indicate that the transient increase in DA outflow evoked by A β was mediated by activation of $\alpha 7^*$ nAChRs.

3. Discussion

It has been proposed that under normal conditions soluble A β has a physiological function (Kamenetz et al., 2003; Wilquet and De Strooper, 2004; Pearson and Peers, 2006). As A β accumulates during the course of AD, however, it likely disrupts neuronal signaling in a variety of ways, dependent upon its actual form (monomeric, small or large oligomeric, aggregated, protofibrillar, fibrillar) (eg. Ye et al., 2004; Bell et al., 2004). That accumulation of soluble A β is relevant to AD is supported by the strong correlation of soluble A β levels with neuropathology in AD patients (Lue et al., 1999; McLean et al., 1999). It was previously demonstrated that acute application of relatively low concentrations (pM-nM) soluble A β can induce increases in presynaptic Ca²⁺ level in a manner that appears to involve nAChRs (Dougherty et al., 2003). Freshly made A β solutions at pM to nM final concentrations will result largely in the formation of small oligomers (Klein, 2002; Chromy et al., 2003; Gong et al., 2003; Stine et al., 2003; Bell et al., 2004). In the present study, acute application of soluble A β into mouse prefrontal cortex was shown to increase transiently the release of DA, measured as overflow via microdialysis in the presence of TTX, whereas control peptides were without effect. A core fragment comprising residues 12-28 of A β (see Wang et al., 2000a) also stimulated DA release but the effect was variable.

The stimulatory effect of A β occurred through an action on presynaptic $\alpha 7^*$ nAChRs, as based on the block of the stimulatory effect by α -bungarotoxin (Fig. 2B) and the loss of the stimulatory effect in $\alpha 7$ null mutant mice (Fig. 3A). In addition, very low relative concentrations (pM) of soluble A β caused a slowly developing, long-lived depression in DA in the prefrontal cortex. In view of the lack of this inhibited phase of DA release in $\beta 2$ null mice (Fig. 3B) or mice treated with a high concentration of a nicotinic antagonist (MLA in Fig. 2B), the possibility exists that the slowly developing depression of DA outflow involves $\beta 2$ containing nAChRs. Interestingly, the apparent EC₅₀ for the stimulation of presynaptic Ca²⁺ by A β was in the pM range (Dougherty et al., 2003), at which only the inhibited phase of DA release was evident (Fig. 2D). Thus, pM A β via presynaptic nAChRs may evoke a sustained but low level increase in presynaptic Ca²⁺, leading to synaptic depression. On the other hand, nicotine perfusion did not evoke this inhibited phase of DA outflow under any condition, nor did nicotine alter the inhibited phase. Thus, alternative targets may exist. Based on previous studies of the effects of soluble A β on synaptic plasticity, possible additional candidate targets might include metabotropic glutamate receptors (Wang et al., 2004) and the nitric oxide pathway (Puzzo et al., 2005; Trabace et al., 2007). Whether the profound decrease in DA in response to very low

concentrations of A β represents synaptic depression also remains to be uncovered. Previous evidence has implicated A β in synaptic depression under a negative feedback system for activity-dependent APP processing in rat hippocampus in a manner dependent upon glutamatergic receptors (Kamenetz et al., 2003). In separate studies, depression of synaptic transmission by A β or A β fragments (eg. A β ₂₅₋₃₅) has been linked to inhibition of voltage-gated Ca²⁺ channels (Ashenafi et al., 2005; Santos-Torres et al., 2007) and reduction of AMPA receptor currents (Hsieh et al., 2006; Shemer et al., 2006; Ting et al., 2007). Furthermore, as soluble A β levels rise in AD, stimulation of DA release might also lead to synaptic depression by depletion over the long run, dependent on the cholinergic activity at the presynaptic nAChRs as well as nerve activity (Dougherty et al., 2003; Kamenetz et al., 2003). It remains to be elucidated whether these effects are pathological or are part of a physiological action of A β . It is very likely that this distinction depends critically on the concentration of soluble A β .

The question arises as to the impact of chronic A β on presynaptic nAChRs. It was previously reported that $\alpha 7^*$ nAChRs are up-regulated in a mouse model for AD, Tg2576, at 9 months of age and then decline thereafter (Jones et al., 2006; however see Oddo and LaFerla, 2006). In preliminary experiments using a model AD mouse at 9 months of age, the DA outflow in response to A β was substantially increased (~3-fold) as compared to age-matched controls (Wu and Nichols, unpublished observations). The extent to which alteration of DA outflow in response to A β reflects up-regulation of nAChRs would need to be determined. The findings presented here would be consistent with the possibility that A β , shown to act as a ligand for $\alpha 7^*$ nAChRs, induces up-regulation when chronically present. In AD, and in older AD mice, the $\alpha 7^*$ nAChRs decrease (Guan et al., 2000; Oddo et al., 2005), suggesting that the system ultimately undergoes degeneration, due most likely to synapse and cell loss.

The prefrontal cortex is implicated in higher executive functions (eg. attention, planning, impulse control), with a prominent role for DA (Arnsten and Li, 2005). The dopaminergic pathway, in turn, is modulated by, among others, nicotine (George et al., 2000). Interestingly, high levels of DA, released, for example, in response to stress, worsen prefrontal cortex function, as gauged by attentional performance (Granon et al., 2000). Thus, normal tonic levels of DA in prefrontal cortex likely act to enhance synaptic function (eg. Matsuda et al., 2006). On the other hand, excessive DA release will disrupt prefrontal cortex synaptic function. The results of the present study suggest that the induction of DA release by A β may alter synaptic function in prefrontal cortex.

4. Experimental Procedures

4.1 Animal Surgery

Adult male C57BL/6J mice (Jackson), C57B1/6 mice harboring a null mutation for the nAChR $\alpha 7$ subunit (Orr-Urtreger et al., 1997) or C57B1/6 mice harboring a null mutation for the nAChR $\beta 2$ subunit (Picciotto et al., 1995) were deeply anesthetized with 60mg/kg *i.p.* pentobarbital (Henry Schein). Under sterile conditions, the anesthetized animals were placed into a Kopf rodent stereotaxic apparatus designed for use with mice and a guide cannula (CMA7) for the microdialysis probe was inserted into the prefrontal cortex, using 10° angle, at 2.0mm anterior to bregma, 1.0mm lateral and -3.0mm ventral from dura (Ihalainen et al., 1999; Paxinos and Franklin, 2001). The probe position and size were chosen to minimize overflow from other structures. (The position of the probe was verified at the end of every experiment (example shown in supplemental Fig. S1).) In the case of perfusion with α -bungarotoxin (Calbiochem), a second, open cannula was cemented next to and slightly above the microdialysis probe cannula, implanting both during surgery, as the toxin will not pass through the microdialysis probe membrane. (In preliminary experiments, perfusion of β -amyloid via the second cannula was also performed, but yielded equivalent results to those obtained wherein β -amyloid was delivered via the microdialysis probe (unpublished findings).)

Animals were kept on an isothermal pad (Deltaphase) during recovery, while bupivacaine (0.5% solution; Henry Schein) was applied liberally to the surgical site during and after recovery. The surgical procedure followed a protocol approved by the Drexel University College of Medicine Institutional Animal Care and Use Committee. A colony of mice harboring a null mutation for the nAChR $\alpha 7$ subunit was established from heterozygous breeders obtained courtesy of Dr. Michael Marks, University of Colorado. A colony of mice harboring a null mutation for the nAChR $\beta 2$ subunit was established from heterozygous breeders obtained courtesy of Dr. Marina Picciotto, Yale University. Routine genotyping of offspring was performed to identify homozygous null mutants ($\alpha 7^{-/-}$ and $\beta 2^{-/-}$).

4.2 In Vivo Microdialysis

Between 18-24h after surgery, the holder insert in the cannula was removed and the microdialysis probe (CMA11, membrane length: 1 mm) was inserted. An output dialysis line from a microdialysis pump (CMA/102) was attached via a freely rotating swivel assembly (BAS) to the microdialysis probe of cannulated mice. The mice were maintained in a plastic cage where they could freely move and have access to food and water during the course of the experiment. Perfusion with sterile artificial cerebrospinal fluid (aCSF) composed of 145mM NaCl, 2.8mM KCl, 1.2mM CaCl_2 , 1.2mM MgCl_2 , 0.25mM ascorbate, 5mM glucose plus 20 mM Na-phosphate, pH 7.4 (He and Shippenburg, 2000) was commenced at 1 $\mu\text{l}/\text{min}$. After 1-2h, sampling of the perfusate was started, collecting fractions into perchloric acid in tubes in a microfraction collector (CMA/142), typically every 20min. After collecting three fractions to determine basal outflow, perfusion with 1 μM tetrodotoxin (TTX; Calbiochem) in sterile aCSF was initiated 10 min prior to perfusion with stimulatory agents (in TTX/aCSF). Even though nM TTX is sufficient to block action potentials in isolated preparations, the use of 1 μM TTX is necessary to ensure full block (Boehnke and Rasmusson, 2001) without noted risk to the mice. Where antagonists were used, they were included during the TTX preincubation period. β -amyloid peptides (Bachem) were solubilized in aCSF immediately before use, as described previously (Dougherty et al., 2003). Fractions were analyzed on an ESA Coulochem II HPLC equipped with a 3 μm -particle MD150X2 column (mobile phase: ESA MD-TM (acetonitrile: octanesulfonate/EDTA/TEA/Na $\cdot$ phosphate in HPLC-grade water, pH 3) at 0.5ml/min) and a high-sensitivity electrochemical detector (guard cell: 350mV; analytical cell: 300mV detection potential; 0.05-0.1 pg/ μl sensitivity for dopamine). Dopamine (DA) standards were injected into the HPLC before assaying the dialysates in order to determine DA retention time. Mean basal levels of DA (pg/ μl) were determined for the first 3 fractions (basal) based on regression analysis of the areas under the DA peaks relative to DA standards, as adjusted for probe recovery (Wang et al., 2000b; Knobelmann et al., 2001). Release of DA in response to applied agents is expressed as a percentage of the basal level in order to normalize responses across animals.

4.3 Immunostaining

To assess perfusion of A β into the prefrontal cortical tissue surrounding the probe, microdialysis with 100nM A β_{1-42} was performed as described in the previous section for 30 min. The probe and guide cannula were immediately removed and India ink was quickly injected into the hole formerly occupied by the cannula to mark the probe site. The mouse was euthanized and the intact brain was removed to an ice-cold glass plate. The area of interest was sliced out, using the dye as a guide. The brain area was then fixed in 4% paraformaldehyde in 0.1 M sodium phosphate-buffered saline (PBS) for 2h. The slice was then transferred to 20% sucrose in PBS for cryoprotection for 15h at 4°C, sectioned on a freezing microtome at 40 μm and mounted on glass slides. The sections were incubated or not (control) for 40h at 4°C in anti- β amyloid monoclonal antibody (6E10; Sigma) at 1:400 dilution in 0.1 M PBS containing 4% bovine serum albumin and 0.3% Triton X-100 (PBS-BSA-T). The sections were then washed three times in PBS for 10min each, incubated with FITC-conjugated affinity purified

goat anti-mouse IgG (1:200; Jackson ImmunoResearch) in PBS-BSA-T for 2 h, washed with PBS and finally coverslipped, sealing with nail polish. Sections were imaged via confocal microscopy. Sections incubated without primary antibody (control) were imaged first, to set range for the baseline “black-level” on the confocal imaging system for background staining due to the secondary antibody alone (see Wu et al., 2006).

4.4 Statistics

Most experiments were replicated 3-6 times. Where indicated, the significance of the difference between mean values was determined by one-way ANOVA followed by Scheffé's F test. Differences were considered significant when p was minimally <0.05 .

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

The work was supported by grants from the NIH (AG21586) and the State of Pennsylvania Tobacco Formula Funds. We thank Ms. Catherine Choi and Ms. Tejal Mehta for help in establishing the colonies of transgenic mice.

Abbreviations

A β , β -amyloid; aCSF, artificial cerebrospinal fluid; AD, Alzheimer's disease; APP, amyloid precursor protein; BgTx, α -bungarotoxin; DA, dopamine; DHBE, dihydro- β -erythroidine; HBS, HEPES-buffered saline; MLA, methyllycaconitine; nAChRs, nicotinic acetylcholine receptors; 3xTg-AD, triple-transgenic AD mice; TTX, tetrodotoxin.

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Fig. 1.

Microdialysis perfusion of β -amyloid. β -amyloid perfused into surrounding tissue via the microdialysis probe (*blank areas along right side of micrographs) placed in the prefrontal cortex for 30 min was detected via immunocytochemistry, as described in Experimental Procedures, using an anti-A β monoclonal antibody (right) in comparison to a control section incubated with fluorescein-conjugated secondary antibody alone (left). Images of 40 μ m sections around the microdialysis probe (*) were taken using confocal microscopy. Scale bar = 12 μ m. Posthoc staining with Hoescht did not reveal any gross alteration in the tissue following A β perfusion (not shown).

Fig. 2.

Nicotine and β -amyloid evoke increased DA outflow in prefrontal cortex. (A) Effect of perfusing 1 μ M nicotine by “reverse” dialysis into prefrontal cortex of freely moving C57B1/6 mice on the release of DA was assessed via *in vivo* microdialysis in the presence of TTX. The perfusate was switched to aCSF containing 1 μ M TTX (as control), TTX plus 1 μ M nicotine or TTX plus 1 μ M nicotine plus 2 mM MLA for 5 min, after which the perfusate was switched by to aCSF containing TTX alone. Under the time frame used for drug perfusion (1–3 h), TTX alone did not significantly affect DA outflow. After extended perfusion (4–6 h), the DA outflow dropped substantially (not shown). MLA was used a general nAChR antagonist, as its use at 2 mM will block both $\alpha 7$ and non- $\alpha 7$ nAChRs (Ward et al., 1990; Mogg et al., 2002). (B) Perfusion with 100 nM A β ₁₋₄₂ in aCSF in TTX for 20 min in the absence or presence of BgTx (1 μ M) or MLA (1 μ M), also delivered for 20 min. BgTx was delivered via a second, open cannula, implanted next to and slightly above the microdialysis probe. (C) Perfusion with 100 nM A β ₁₂₋₂₈ in TTX, a highly soluble core fragment which strongly competes for A β ₁₋₄₂ interaction with $\alpha 7$ nAChRs (Wang et al., 2000a). (D) Perfusion with various concentrations of A β ₁₋₄₂ in TTX. (E) Perfusion with the peptide “A β ₄₀₋₄₁” in TTX, which served as a control “reverse” peptide having no effect on presynaptic Ca²⁺ (Dougherty et al., 2003). (Lack of effect of “A β ₄₂₋₁” on presynaptic Ca²⁺ has also been observed (Mehta et al., manuscript in

preparation).) Dopamine (DA) content in the fractions (preloaded with perchloric acid) was determined via HPLC with an electrochemical detector, as described in the Experimental Procedures. Data are presented as averages $\pm$ s.e.m (4-6 replicates each). Mean basal values for DA: 0.5-1pg/ul, adjusted for recovery (4-10%); * p <0.05 relative to baseline.

Fig. 3.

β -amyloid-evoked DA outflow in prefrontal cortex of mice harboring null mutations of nAChR subunits. (A) Perfusion with 100pM or 100nM $A\beta_{1-42}$ for 5min into prefrontal cortex of $\alpha 7$ nAChR null-mutant mice (Alpha7 KO). (B) Perfusion with 100nM $A\beta_{1-42}$ into prefrontal cortex of $\beta 2$ nAChR null-mutant mice (Beta2 KO). Shorter perfusion with $A\beta$ in A and B was performed in order to reveal possible slowly developing inhibition of DA outflow resulting from the fall-off in $A\beta$ following perfusion. (C) Perfusion with 1 μ M nicotine for 30min, then 100nM or 10pM $A\beta_{1-42}$ with nicotine for an additional 20min. Experiments were performed as described in the legend to Fig. 2. Data are presented as averages $\pm$ s.e.m; $n=3-5$ replicates

each, except in C where only averages are shown for clarity ($n=3$). Mean basal values for DA: 0.5-1pg/ul, adjusted for recovery (8-23%); $*p<0.05$ relative to baseline.