JPET#84764

# Effects of β-phenylethylamine on Dopaminergic Neurons of the Ventral Tegmental Area in the Rat: A Combined Electrophysiological and Microdialysis study

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JPET Fast Forward. Published on May 5, 2005 as DOI: 10.1124/jpet.105.084764
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a) Running title: β-phenylethylamine and dopamine neurons in rat VTA

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c) Number of text pages: 20
Number of figures: 7
Number of tables: 0
Number of references: 35
Number of words in Abstract: 197
Number of words in Introduction: 399
Number of words in Discussion: 1132

d) Abbreviations: β-PEA, β-phenylethylamine; VTA, ventral tegmental area; DA, dopamine; HPLC , high-performance liquid chromatography; PTX, Pertussis toxin;

e) Neuropharmacology

**ABSTRACT**

The effects of systemic administration of β-phenylethylamine (β-PEA) and microiontophoretically applied β-PEA on the spontaneous discharge of dopamine (DA) neurons in the ventral tegmental area (VTA) of the anesthetized rat were examined. Intravenous administration of β-PEA (1.0, 2.5, 5.0 mg/kg) and microiontophoretic applications of β-PEA caused inhibitory responses in DA neurons. Systemic administration and microiontophoretic applications of β-PEA induced dose- or current-dependent responses. The systemic β-PEA-induced inhibitory responses were reversed by pretreatment with the DA $D_2$ receptor antagonists, haloperidol (0.5 mg/kg, i.p) and sulpiride (10 mg/kg, i.p). Pretreatment with reserpine (5 mg/kg, i.p., 24 h earlier) did not completely block the systemic administration of β-PEA (2.5 mg/kg) inhibition. A microdialysis study of freely moving rats demonstrated that the extracellular DA level increased significantly in response to local application of β-PEA (100 mM) in the VTA via a microdialysis probe, and local application of β-PEA stimulated somatodenderitic DA release in the VTA. The β-PEA-induced release of DA was calcium ion–independent and was enhanced by pretreatment with pertussis toxin. These findings indicate that β-phenylethylamine inhibits DA neuron activity via DA $D_2$ autoreceptors in the rat VTA and that this inhibitory effect is mediated by the somatodendritic DA release.

## Introduction

β-phenylethylamine (β-PEA) is an endogenous trace amine synthesized by decarboxylation of phenylalamine in dopaminergic neurons of the nigrostriatal system (Wu and Boulton, 1975; Dyck et al., 1983; Juorio et al., 1991). The existence of β-PEA is well established in the central nervous system. The highest brain levels of β-PEA have been found in the mesolimbic area, caudate-putamen structures and hypothalamus (Durden et al., 1973; Philips et al., 1978). β-PEA is present in tyrosinehydroxylase-containing neurons and coexists with dopamine (DA) in the nigrostriatal pathways (Juorio et al, 1991). Altered endogenous β-PEA levels in the plasma of stressed or schizophrenic patients have been previously reported (Paulos and Tessel 1982; Szymanski et al., 1987).

The effects of β-PEA have been assessed electrophysiologically in a number of different areas of the brain. For example, microiontophoretic application of β-PEA acts post-synaptically to potentiate the cortical neuron response to iontophoretically applied norepinephrine (Paterson and Boulton, 1988) and the caudate nucleus neuron response to iontophoretically applied DA (Paterson et al., 1990). Moreover, DA neurons in the rat substantia nigra pars compacta are inhibited by systemic administration of β-PEA (Rodriguez and Barroso, 1995). In biochemical studies, systemic application of β-PEA stimulates the release of DA in the striatum (Dyck et al., 1983; Philips, 1986; Bailey et al., 1987) and postsynaptic DA receptors (Antelman et al., 1977). Because β-PEA alters several neurotransmitter systems (Sloviter et al., 1980; Kato et al., 2001) and stimulates the release of DA, dopaminergic neurons could be involved in β-PEA-induced behaviors (Barroso and Rodriguez, 1996). An in vivo microdialysis study indicated that locally applied β-PEA directly produced a dose-dependent increase in extracellar DA release in the nucleus accumbens (Nakamura et al., 1998). Recently, Geracitano et al. (2004) demonstrated that β-PEA inhibits the activity of dopaminergic neurons in the substantia nigra and ventral tegmental area (VTA) using intracellular electrophysiological recording.

The data from these studies suggest that β-PEA affects the activity of dopaminergic neurons in the VTA. It is this group of neurons that innervates the mesolimbic and mesocortical areas of the brain, which are important regions in schizophrenia. The present study was designed to further evaluate the response of dopaminergic neurons in the rat VTA to systemic and iontophoretic administrationof β-PEA. In addition, in vivo microdialysis was employed in an effort to ascertain whether the actions of β-PEA in this region wre mediated by release of somatodendric DA in the VA.

## Materials and methods

**Animals.** Male Wistar rats were housed individually under automatically controlled environmental conditions and a 12-h light-dark cycle with free access to food and water. All animals were quarantined in centralized animal facilities for at least seven days upon arrival. Each animal was used only once in the present study. Experiments were carried out according to the guidelines for animal care and use published by the National Institutes of Health and Showa Pharmaceutical University.

**Surgical Procedures Used in Electrophysiological Study.** Male Wistar rats, weighing between 270 and 350 g, were anesthetized with chloral hydrate (400 mg/kg, i.p. plus a supplementary dose of approx. 60 mg/kg/h). The femoral artery and vein were cannulated to enable continuous recording of arterial blood pressure and intravenous administration of drugs. A local anesthetic (8% lidocaine spray) was applied to all wound margins. The rat heads were placed in stereotaxic frames. Body temperature, which was continuously monitored by rectal probe, was maintained at 37$^{o}$C by means of a heating pad. A second group of animals was also pretreated with reserpine (5 mg/kg, i.p.) 24 h before the experiments.

**Single cell Recordings and Microiontophoresis.** The rat heads were mounted in a

stereotaxic apparatus with a nose bar setting of -3.3 mm below the interaural zero. A burr hole was then made in the skull over the ventral tegmental area and the dura incised. The coordinates for locating the VTA were translated into distance (mm) from the bregma (-5.5 to -5.8), the midline suture (0.3 to 0.5) and depth below the dura (-8.0 to -9.0) (Paxinos and Watson, 1986). Extracellular recordings were made with single glass capillaries pulled and bumped (under microscopic control) to a tip diameter of approx. 1 μm and filled with a solution of 3 M NaCl containing 3% Pontamine sky blue, to mark recording sites. The microelectrode was lowered by a digital readout micromanipulator (Narishige, Tokyo, Japan). Single unit spontaneous activity was amplified, displayed on a medical oscilloscope with an audiomonitor and separated from the background noise (bandpass filtering 3 kHz) by a voltage gating window discriminator. A signal processor (Model 7T08, Nihondenki Sa-ei Instrument Co., Ltd, Tokyo, Japan) was used for compiling the data in the form of rate histograms.

Four-barreled glass micropipettes were used. The tip of the micropipette was broken back to a diameter of approximately 4~7 μm, under microscopic control. The recording barrel was filled with a 3 M NaCl solution containing 5% fast green dye to mark recording sites (resistance 10~20 MΩ). Three of the four side barrels were filled with the following drugs; 0.1 M dopamine hydrochloride, pH 4.5 or 0.1 M β-phenylethylamine hydrochloride, pH 4.5. The remaining side barrel, the balance barrel, was filled with a solution of 2 M NaCl. Substances were applied from the micropipettes adjacent to the recording site by microiontophoretic application, which was performed using a constant-current pump (Iontophoresis Pump Neuro Phore BH-2, Medical System Co., USA), with a 10~15 nA retaining current. All substances were injected as cations.

The spontaneous activity of each neuron was monitored in the first instance for 5-10 min to ensure a stable baseline. The effects of the previous drug on the spontaneous discharge were observed after ejection for 60 sec. The next drug was ejected 1-3 min after recovery of the responses. After all the experiments were completed, the position of the

electrode tip on the VTA was verified. A negative current of 20~40 nA was applied for 15~20 min through the central barrel, which was filled with fast green dye. Animals were sacrificed by intravenous injection of pentobarbital-Na and perfused through the heart with PBS followed by 10% formalin and isotonic saline. Several days later, the brain coronal slices (30 mm) were stained with cresyl violet, and electrode placement in the VTA was determined by light microscopy.

**Identification of Putative Dopamine Neurons.** DA neurons were established electrophysiological characteristics, as previously described (Bunney et al., 1973; Wang, 1981). These included: (1) a long action potential duration (>2 msec) with a distinct "notch" during the initial rising phase; (2) a biphasic action potential (positive-negative waveform); (3) slow, burst, or regular firing pattern (0-9 spikes/sec).

**Surgical Procedures Used in the *in vivo* Microdialysis Study.** Male Wistar rats weighing 260 and 310 g were anesthetized with pentobarbital-Na (50 mg/kg, i.p.), and positioned in a stereotaxic apparatus. Each rat's skull was exposed and drilled for implantation of a guide canulla into the upper part (2.0 mm) of the VTA (bregma: -5.5 mm, lateral: 0.4 ~ 0.6 mm, depth: -6.5 ~ -8.5 mm, Paxinos and Watson, 1986). The guide canulla was held firmly in place by dental acrylic and anchored to the skull using stainless screws. All experiments were performed 6 days after surgery.

***In vivo* Microdialysis.** Microdialysis probes (dialysis membrane: length 2.0 mm, diameter 0.3 mm, mol/wt. cut-off 20,000; AF-01, Eicom Co., Japan) were inserted into the VTA through the previously implanted guide canulla. The animals were placed in a Plexiglas cage (30 cm×30 cm×38 cm), and were connected by polyethylene inflow and outflow tubes to a syringe pump (CMA 100; Carnegie Medicine) and collection vials. Perfusion solution (125 mM NaCl, 3 mM KCl, 1.3 mM $CaCl_2$, 1 mM $MgCl_2$ and 23 mM $NaHCO_3$) in

aqueous potassium phosphate buffer (1 mM, pH 7.4) was perfused into the dialysis probe at a rate of 2 mL/min. The perfusate was collected at 20 min intervals. DA was quantitatively measured in each perfusate using high-performance liquid chromatography (HPLC) with electrochemical detection (HPLC-ECD, HTEC-500 system, Eicom Co., Kyoto, Japan). The HPLC system and conditions were as follows: Eicom EP-300/Eicom ECD-300 system; column, Eicompak SC-5ODS (3.0 mm i.d.¥150 mm) with precolumn; mobile phase, 85% 0.1 M citric acid/ 0.1 M sodium acetate buffer, 15% methanol, 0.023% sodium 1-octanesulfonate, containing 5 mg/l disodium-EDTA; flow rate, 1 ml/min; electrode, Eicom WE-3G graphite electrode; reference electrode, Eicom RE-100 Ag/AgCl; applied voltage, 650 mV vs. Ag/AgCl. Column temperature was maintained at 25$^{o}$C.

After all the experiments were completed, the position of the probe in the VTA was verified. Animals were sacrificed by intravenous injection of pentobarbital-Na and perfused through the heart with PBS followed by 10% formalin and isotonic saline. Several days later, the brain coronal slices (60 mm) were stained with cresyl violet, and probe placement in the VTA was determined by light microscopy. Rats were included in data analysis only if at least 80% of the active region of the microdialysis membrane was located within the anatomical borders of VTA. VTA probes were located between lateral 0.5~0.9 mm and depth -8.5 ~-9.0 mm. Any rat with the VTA probe >1.0 mm lateral to the midline was not included.

**Drugs.** β-PEA was purchased from Tokyo Chemical Industry Co. (Tokyo, Japan) and was dissolved in physiological saline for intravenous administration. Japan. Sulpiride was purchased from Fujisawa, Osaka, Japan. Haloperidol and Reserpine were purchased from Daiichi Seiyaku, Osaka, Japan. Dopamine hydrochloride and Pertussis toxin (PTX) were purchased from Sigma (St. Louis, MO. USA).

β-PEA was dissolved in perfusion solution for VTA application through the dialysis probe. Analytical grade chemicals for perfusion solution and mobile phase were from

Tokyo Chemical Industry Co. (Tokyo, Japan) or Wako Pure Chemical Industries (Osaka, Japan).

**Statistical Analysis.** The results of the microdialysis study are expressed as means±S.E.M. of the amount of DA in VTA perfusates. Statistical significance was analyzed by non-paired t-test or one-way repeated measurement analysis of variance (RM-ANOVA), followed by Dunnett's multiple comparison test. Data were analyzed with StatView software for Macintosh. Differences were considered significant when P value was less than 0.05.

## Results

**Effects of Systemic β-phenylethylamine on Putative Dopamine Neurons of VTA**. Single-unit extracellular recordings were made from putative DA neurons identified by electrophysiological characteristics. Putative DA neurons had regular or slow bursting patterns of 0.4 to 9.7 spikes/sec and an average firing rate of 4.9±1.5 spikes/sec (mean± S.E.M). The responses of 25 putative DA neurons were measured after intravenous administration of β-PEA (1.0, 2.5 and 5.0 mg/kg). Systemic β-PEA consistently produced a dose-dependent inhibition of the firing rate of putative DA neurons (Fig. 1). The inhibition by β-PEA was instantaneous in onset and short lasting - recovery was observed within 5 min. β-PEA (1.0, 2.5 and 5.0 mg/kg) inhibited 35.4±10.2% (n=6), 50.3±12.9% (n=6) and 79.1±15.5% (n=6), respectively. We next examined the effect of pretreatment with haloperidol and sulpiride on β-PEA-induced inhibition of DA neurons in the VTA. The inhibition by β-PEA (2.5 mg/kg) was reversed by pretreatment with the DA $D_2$ receptor antagonists, sulpiride (10 mg/kg, i.p.) and haloperidol (0.5 mg/kg, i.p.) (Fig. 2A and 2B). β-PEA-induced inhibition was reversed in six out of seven neurons, but inhibition of one out of seven neurons was not reversed by haloperidol. Sulpiride, a DA $D_2$ receptor antagonist, reversed β-PEA-induced inhibition in all six DA neurons.

Pretreatment with reserpine (5 mg/kg, i.p., 24 h earlier, n=6 ) did not completely block the β-PEA (2.5 mg/kg) inhibition (Fig. 3).

**Effects of Microiontophoretically Applied β-phenylethylamine on Putative Dopamine Neurons of VTA.** We then examined the effects of microiontophoretically applied β-PEA on spontaneous discharge of putative DA neurons in the VTA. The responses of 16 putative DA neurons to microiontophoretic application of β-PEA in five incremental currents (10, 25, 50, 75 and 100 nA) were investigated. Spontaneous discharges were inhibited in response to microiontophoretic application of β-PEA. Figure 4A is a typical record illustrating the current-dependent inhibition of DA neuron spontaneous discharge by β-PEA applied at 50, 75 and 100 nA. β-PEA inhibited 75.8 ±8.1% (n=6), 88.1 ± 7.4% (n=6) and 92.1 ± 6.3% (n=6) of DA neuron spontaneous discharge at microiontophoretic currents of 50, 75 and 100 nA, respectively. Also, DA inhibited 61.1 ±7.3% (n=4), 85.2 ± 8.1% (n=4) and 92.6 ± 4.8% (n=4) of DA neuron spontaneous discharge at microiontophoretic currents of 50, 75 and 100 nA, respectively. Figure 4B shows current-dependent inhibition of VTA DA neuron spontaneous discharge by β-PEA and DA. The inhibition by β-PEA (50 and 75 nA) was reversed by systemic administration of sulpiride (10 mg/kg) (Fig. 4C). Sulpiride reversed β-PEA-induced inhibition in all four DA neurons.

**Effects of Locally Applied β-phenylethylamine on Dopamine Release in VTA.** We next examined the effects of local application of β-PEA (1, 10 and 100 mM) through the microdialysis probe on DA release in the VTA (Fig. 5A). The basal concentration of DA in control samples was 2.42 ± 0.7 pg /20 min (n=19). As shown in Figure 5A, perfusion of β-PEA (1 mM; n=4) did not affect the release of DA (2.61 ± 0.8 pg /20 min) at 20 min in the VTA. β-PEA (10 mM; n=5) slightly increased the release of DA (3.7 ± 0.8 pg /20 min) in the VTA, but not significantly (n=5). Local application of β-PEA (100 mM; n=5)

significantly increased the release of DA with a maximum effect of 8.1± 0.7 pg/20 min observed at 20 min [one-way RM-ANOVA:$F_{(18,54)}$=10.55, P<0.001, n=5]. The increase in DA release was reversed after removal of β-PEA from the perfusate. Figure 5B shows the effects of β-PEA (1, 10 and 100 mM) on total release of DA over 60 min in the VTA. The dialysate concentrations of DA increased from 6.9 ± 1.4 pg /60 min (total basal release for 60 min) to 8.0 ± 1.4 pg /60 min, 11.1 ± 2.2 pg /60 min and 21.8 ± 3.7 pg /60 min, respectively, at doses of 1, 10 and 100 mM of β-PEA (Fig. 5B). Local application of β-PEA (100 mM), for 60 min significantly increased the release of DA in the VTA (Dunnett's multiple comparison test: P<0.001. Fig. 5B).

**Calcium-Independence of Increased Dopamine Release in VTA in Response to Locally Applied β-phenylethylamine**. We examined the effects of local application of β-PEA (100 mM) and calcium ion free solution on DA release in the VTA. Calcium ions were omitted from the perfusate, and 0.5 mM EGTA was added to determine the dependence on extracellular calcium ions. No significant differences were observed in β-PEA (100 mM) induced total DA release for 60 min between samples perfused with calcium ions (22.1 ± 1.2 pg /60 min) or without calcium ions (21.0 ± 1.3 pg /60 min) in the VTA (non-paired t-test: t(6)=0.084, P=0.936, n=4) (Fig. 6). Local application of β-PEA (100 mM) did not affect the release of DA in the VTA.

**Effects of Pretreatment with PTX on Dopamine Release in VTA in Response to Locally Applied β-phenylethylamine.** Finally, we examined the effects of pretreatment with PTX on DA release in the VTA in response to locally applied β-PEA. To examine the involvement of intracellular signal transduction in the β-PEA-induced increase in DA release, PTX (1.0 mg/ml, 0.5 mg/site, n=4) or vehicle (n=4) was injected into the ipsilateral VTA through a microsyringe 8 days before the local application of β-PEA (10 and 100 mM). PTX treatment alone had no significant effect on the basal DA (2.5 ± 0.8

pg /20 min) release. No significant differences were observed in β-PEA (10 mM) induced total DA release over 60 min between non-treated samples (7.7 ± 1.5 pg /60 min, n=4) and PTX-treated (11.3 ± 1.8 pg /60 min, n=4) samples in the VTA (non-paired t-test: t(6)=0.270, P=0.769) (Fig. 7). However, DA release induced by 100 mM β-PEA significantly increased from 22.5 ± 1.8 pg /60 min (n=4) to 31.8± 2.7 pg /60 min (n=4) in response to pretreatment with PTX (non-paired t-test: t(6)=3.386, P=0.015) (Fig. 7).

## Discussion

**Dopamine Neuron Activity is Inhibited after β-Phenylethylamine Administration.** In the present electrophysiological study, we observed that systemic administration of β-PEA decreased the spontaneous firing rate of DA neurons in the VTA. These results are in agreement with previous reports that systemic β-PEA decreased the firing rate of nigrostriatal dopaminergic neurons in a dose-dependent manner (Rodriguez and Barroso, 1995). The inhibitory effect of systemic β-PEA on DA neurons was blocked by pretreatment with the DA $D_2$ receptor antagonists haloperidol and sulpiride. Similarly, β-PEA decreased dopaminergic neuron activity in an in vitro electrophysiological study of VTA, and this inhibition was blocked by sulpiride (Geracitano et al., 2004). In additional experiments, we found that iontophoretic application of β-PEA and DA directly decreased the activity of DA neurons within the VTA in a current-dependent manner. β-PEA and DA showed similar inhibitory activity on the DA neurons. The decrease produced by iontophoretic application of β-PEA was blocked by sulpiride, indicating that the DA $D_2$ autoreceptor in the VTA is involved in the effects of β-PEA. However, microiontophoretic administration of β-PEA-induced inhibition via the DA $D_2$ receptor could not be determined from the present results, since $D_2$ receptor antagonist sulpiride was administrated systemically. A recent in vitro electrophysiological study demonstrated that inhibitory spontaneous firing induced by β-PEA was mediated by indirect activation of DA $D_2$ autoreceptors caused by an increase in DA release. Moreover, chronic administration of β-

PEA caused down-regulation of DA $D_2$ receptors in rats (Paetsch and Greenshow, 1993), indicating that β-PEA could affect the function of DA $D_2$ receptors. The inhibitory effects of β-PEA on DA neurons appeared to be dependent on stimulation of the DA $D_2$ receptor, since these effects were reversed by the $D_2$ receptor antagonists sulpiride or haloperidol, but not by $a_1$ receptor antagonist prazosin (data not shown). The present results suggested that β-PEA-induced inhibitory responses were affected by activation of DA $D_2$ autoreceptors in the somatodendrites of the VTA. Thus, DA $D_2$ receptor activation elicits hyperpolarization and decreases the firing rate (Bunney et al., 1973; Mercuri et al., 1997) due to activation of an inwardly rectifying $K^+$ condactance (Lacey et al., 1987; Kim et al., 1995). It is possible that β-PEA causes such an inhibitory effect by activating $K^+$- channels, which would produce hyperpolarization of the DA neuron. However, since the transmembrane properties of DA neurons in the presence of β-PEA have yet to determined, these explanations remain speculative at this point.

Our data show that inhibitory responses of dopaminergic neurons induced by systemic β-PEA are not affected by pretreatment with reserpine. In fact, pretreatment with reserpine does not affect the stereotypical or rotational behavior induced by β-PEA (Baud et al., 1985; Barroso and Rodriguez, 1996). Furthermore, β-PEA stimulates cytoplasmic DA release from nigrostriatal neurons (Barroso and Rodriguez, 1996). Together, these results indicate that β-PEA affects dopaminergic neurotransmission by interacting with the reserpine-resistant pool of DA. On the other hand, previous in vivo and in vitro studies have demonstrated that β-PEA inhibits the uptake of DA (Raiteri et al., 1977; Bailey et al., 1987). Iontophoretically applied β-PEA acts post-synaptically to potentiate the cortical neuron response to microiontophoretically applied norepinephrine (Paterson and Boulton, 1988) and the caudate nucleus neuron response to iontophoretically applied DA (Paterson et al., 1990). Therefore, inhibition of the uptake mechanisms by β-PEA may contribute to the increased DA release. Recently, in an in vitro electrophysiological study of β-PEA, membrane changes in dopaminergic neurons in the VTA did not potentiate the inhibitory

responses to exogenously applied DA (Geracitano et al., 2004). Thus, the present data suggest that β-PEA stimulates the release of newly synthesized DA from the cytoplasmic pool in the VTA dopaminergic somatodendrites.

**Dopamine Release Increases after β-Phenyletylamine Application.** Since DA release in the VTA appears to be closely related to the β-PEA-induced inhibitory responses, the question arises as to whether the effects of β-PEA on somatodendric DA release is due to the decrease in VTA dopaminergic activity. Substantial evidence suggests that β-PEA stimulates the release of DA from striatal slices (Dyck, 1983; Yamada et al., 1998) and in cannular perfusate in vivo (Philips and Robson, 1983). This DA release is thought to be from the cytoplasmic DA pool and is carrier-mediated and $Ca^{2+}$-independent (Liang and Rutledge, 1982; McMillen, 1983). Likewise, β-PEA is known to increase the release of DA in the rat striatum (Bailey et al., 1987; Kuroki et al., 1990) and nucleus accumbens (Nakamura et al., 1998). β-PEA regulates dopaminergic neurotransmitter release, and this regulation is especially predominant in dopaminergic neurons. The present study is the first to show that local application of β-PEA into the VTA using in vivo microdialysis increased the extracellular DA release in a dose-dependent manner. Thus, trace amines, such as β-PEA, directly cause an increase in somatodendric DA release in the VTA. It seems likely that the inhibition of spontaneous dopaminergic neuron activity by β-PEA is due to an increased release of DA in the VTA caused by β-PEA. In addition, the β-PEA-induced increase in DA release was completely independent of extracellular calcium ions. β-PEA stimulates in an amphetamine-like manner, causing a $Ca^{2+}$-independent carrier-mediated efflux of DA from the somatodendric dopaminergic neurons in the VTA. Taken together, the results of the present and previous studies suggest that effects of β-PEA on DA release result from a newly synthesized DA and $Ca^{2+}$-independent from dopaminergic somatodendrites in the VTA.

The DA $D_2$ receptor is coupled to the PTX-sensitive G-protein in rat substantia nigra, and PTX treatment blocks DA $D_2$ autoreceptor-mediated inhibition of dopaminergic neurons in the rat substantia nigra (Innis and Aghajanian, 1987). The DA $D_2$ receptor in dendrites is located near the cytoplasmic surface of dendritic plasma membranes where the receptor couples to Gi/o-like proteins, which modulate ion channels and/or depress adenylate cyclase activity (Sesack et al. 1994; Missale et al., 1998). We therefore assessed the role of PTX-sensitive G-protein in the β-PEA-induced increase in VTA DA release. Our results indicated that the increase in DA release induced by local application of β-PEA was significantly enhanced by pretreatment with PTX in the VTA. Thus, enhancement of β-PEA-induced increases in DA release by pretreatment with PTX may result from disinhibition of somatodendric DA $D_2$ autoreceptors in the VTA. The resulting diminished capacity for autoreceptor feedback inhibition of DA release by PTX pretreatment-induced uncoupling of the DA $D_2$ autoreceptors on somatodendritic DA neurons would result in enhanced DA release in the VTA in response to β-PEA. These observations suggest that β-PEA-induced somatodendritic release of DA is regulated by the output of dopaminergic neurons in the VTA, and endogenous β-PEA may play a role in interneuronal VTA communication.

We conclude that β-PEA-induced inhibitory responses could occur via the release of somatodendric DA from the VTA since β-PEA evokes DA release and the inhibitory effect of β-PEA on dopaminergic neurons is regulated by DA $D_2$ somatodendritic autoreceptors.

**Acknowledgments**

The authors gratefully acknowledge Mr. J. Maeda and Mr. K. Takahashi for excellent technical assistance.

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**Footnotes**

The work was supported by the Showa Pharmaceutical University.

**Figure Legends**

Figure 1 Effects of intravenous administration of b-phenylethylamine (β-PEA) on the spontaneous discharge of a putative DA neuron in the rat VTA. Frequency histogram of the firing rate for β-PEA-induced inhibitory response. Note that the effects at each dose of β-PEA (1.0, 2.5 and 5.0 mg/kg, i.v.) resulted in a dose-dependent inhibition of spontaneous activity in the putative DA neuron. The systemic administration of β-PEA is indicated by the arrow. The ordinate shows the firing rate in spikes per second.

Figure 2 Frequency histograms illustrating the effects of pretreatment with sulpiride (10 mg/kg, i.p.) and haloperidol (0.5 mg/kg, i.p.) on putative DA neuron responses to systemic β-phenylethylamine (β-PEA). Pretreatment with sulpiride (A) and haloperidol (B) antagonized the β-PEA (2.5 mg/kg)-induced inhibition.

Figure 3 Frequency histogram illustrating the effects of β-phenylethylamine (β-PEA) on the spontaneous discharge of a putative DA neuron following pretreatment with reserpine (5.0 mg/kg, i.p.; 24 h). The pretreatment with reserpine did not affect the β-PEA (2.5 mg/kg, i.v.)-induced inhibition.

Figure 4 Effects of microiontophoretic application of b-phenylethylamine (β-PEA) on the spontaneous discharge of a putative DA neuron in the rat VTA. A: Frequency histogram of the firing rate for β-PEA-induced inhibitory response. Note that the effects at each dose of β-PEA (50, 75, and 100 nA) resulted in a dose-dependent inhibition of putative DA neuron spontaneous activity. The duration of drug ejection is indicated by the horizontal

bars above each record; numbers directly above each bar refer to the iontophoretic current in nA used for the ejection of β-PEA. B: Effect of microiontophoretic application of DA (10, 25, 50, 75, and 100 nA) and β-PEA (10, 25, 50, 75, and 100 nA) on the current-dependent curve in the VTA DA neuron. C: Pretreatment with sulpiride (10 mg/kg, i.p.) antagonized the microiontophoretic application of β-PEA (50 and 75 nA)-induced inhibition. Furthermore, they were inhibited by microiontophoretically applied dopamine. The abscissa shows the rate of drug ejection. The ordinate shows the firing rate in spikes per second.

Figure 5 A. Effect of locally applied b-phenylethylamine (β-PEA) on DA release from the VTA. β-PEA (1, 10 and 100 mM) was applied into the VTA through the dialysis probe. β-PEA (100 mM) significantly increased the release of DA [one-way RM-ANOVA; $F(18,54)=10.55$, $P<0.001$, $n=5$]. The duration of β-PEA application is indicated by the horizontal bars. B. Dose-response data were transformed into data points that represented the total amount of DA for the 60 min dialysis samples. Asterisk indicates significant differences between DA basal release and β-PEA (100 mM) at $P<0.001$ using Dunnett's multiple comparison test. Data are means ± S.E.M. (bars) of 5 experiments.

Figure 6 Effects of calcium ion on locally applied β-phenylethylamine (β-PEA)-induced increases in DA release from the VTA. A $Ca^{2+}$-free solution containing 0.5 mM EGTA was perfused for 4 h, and β-PEA (100 mM) was administered for 60 min. The increase in DA release in response to locally applied β-PEA (100 mM) was not affected by $Ca^{2+}$-free perfusing solution containing 0.5 mM EGTA. Data are means ± S.E.M. (bars) of four experiments.

Figure 7 Effects of pretreatment with PTX on locally applied, β–PEA-induced increase in DA release from the VTA. PTX (0.5 mg in 0.5 ml of saline) or saline (0.5 ml) was

injected unilaterally into the VTA. After 8 days, the perfusion solution was administered for 200 min and β-PEA (10 and 100 mM) was administered for 60 min, respectively. The increase in the DA release in response to local application of β-PEA (100 mM, n=4) was significantly enhanced by pretreatment with PTX. Data are means ± S.E.M. (bars) of four experiments. Asterisk indicates significant differences between untreated and PTX-pretreated β-PEA (100 mM) samples at $P<0.05$ * by non-paired t-test.

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**Fig. 1**

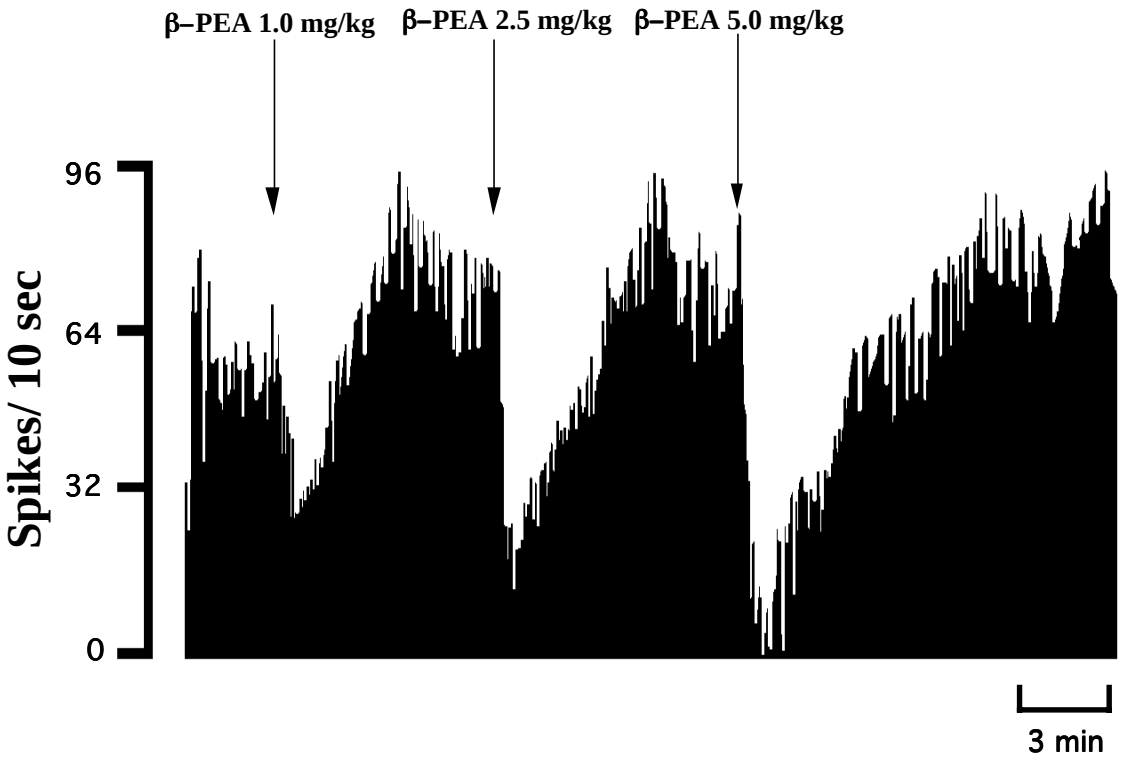

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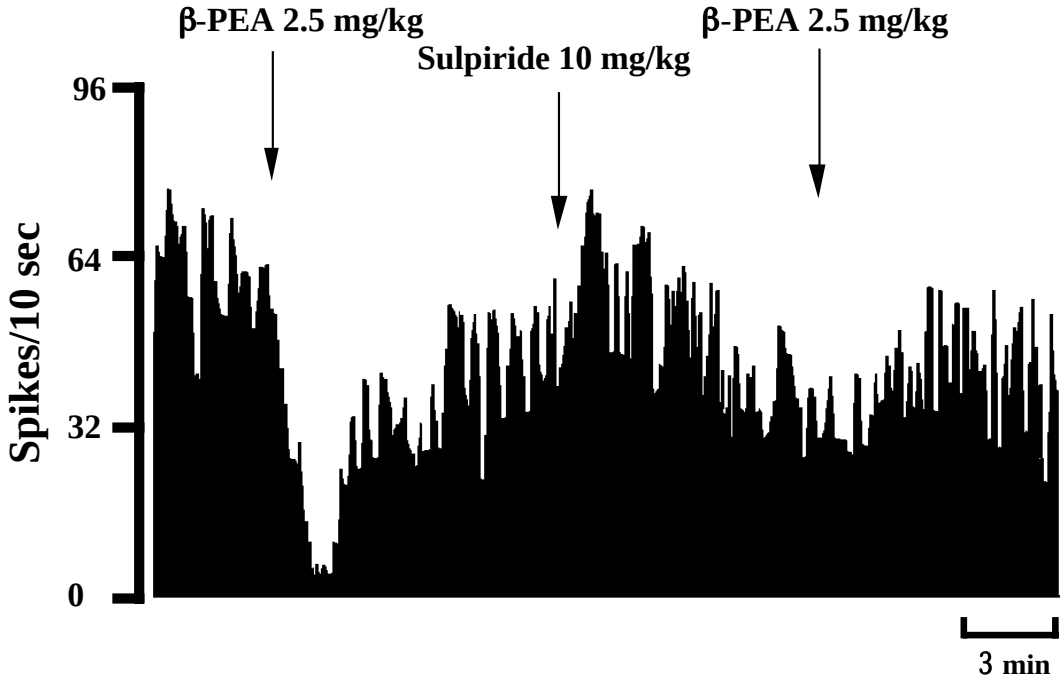
**Fig. 2**

**A**

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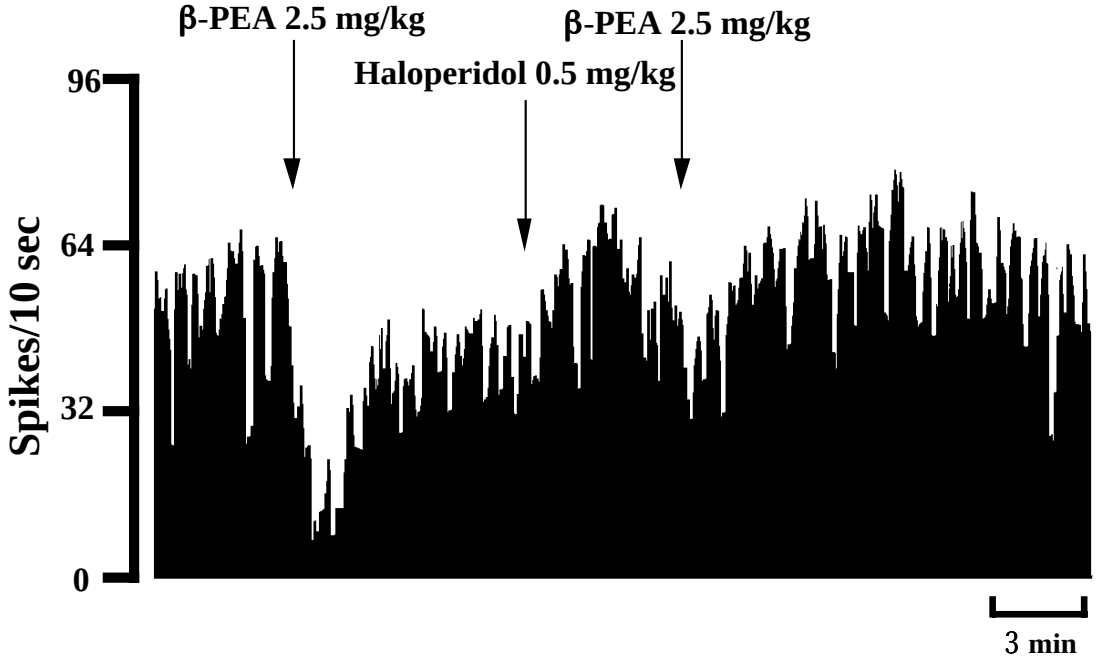

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**Fig.** 3

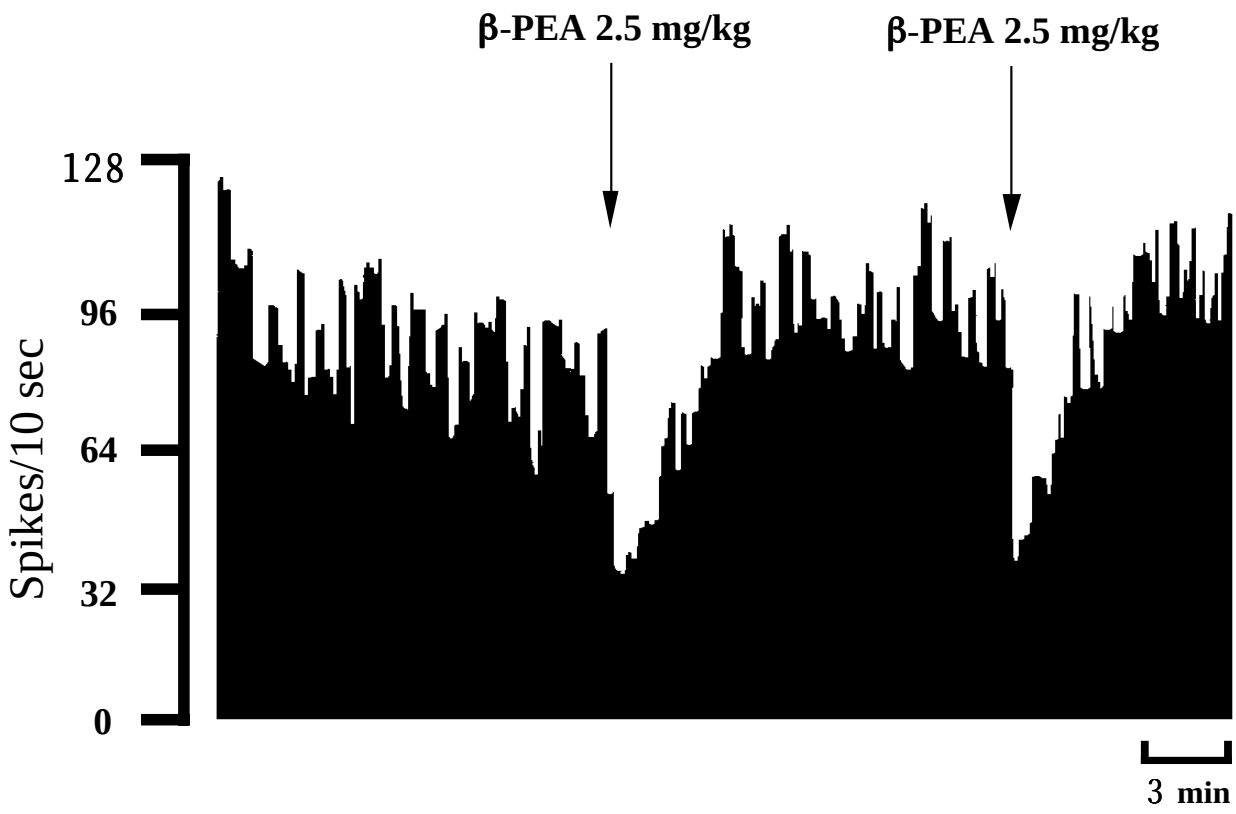

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Fig. 4

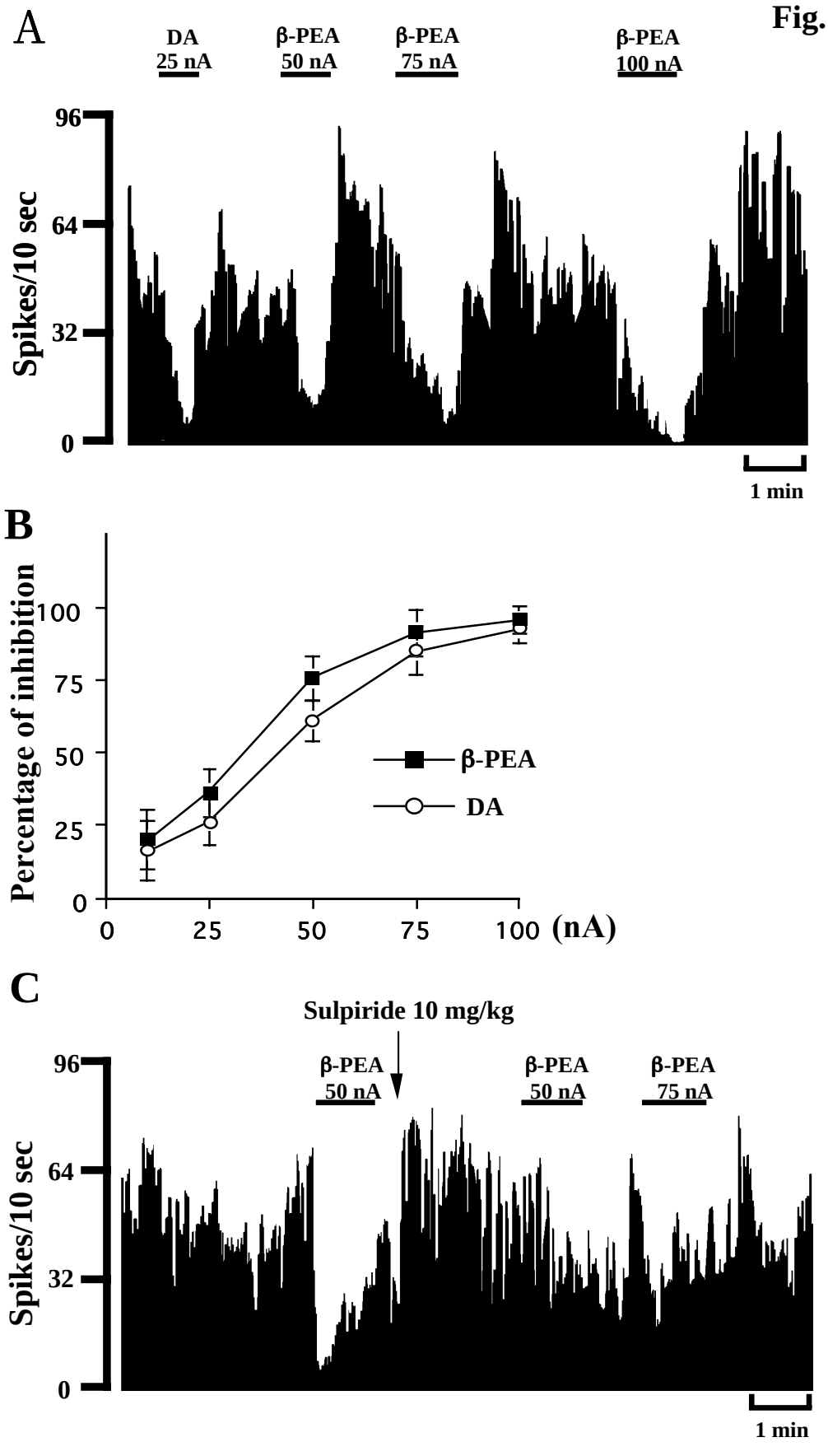

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**Fig. 5**

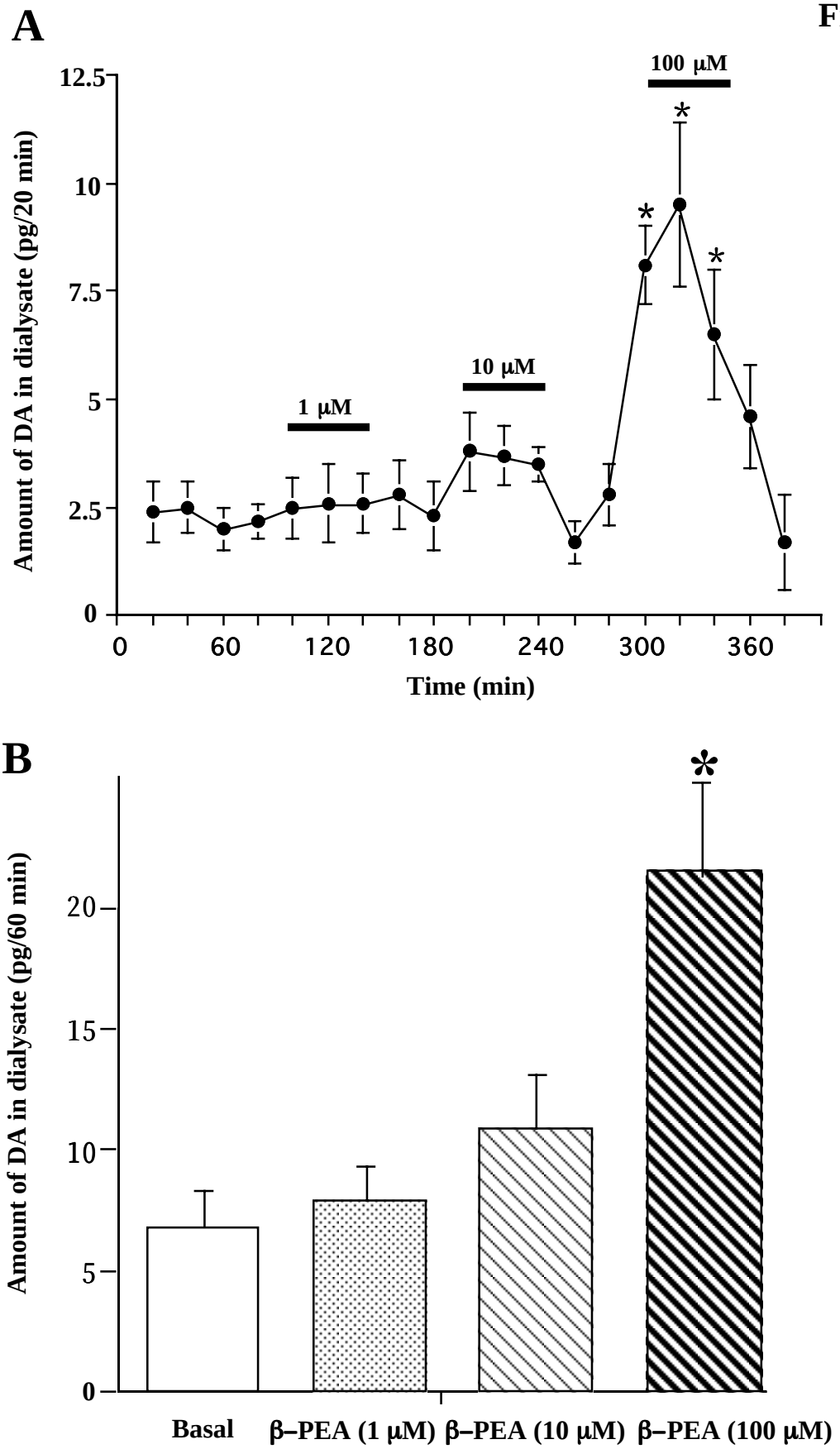

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**Fig.** 6

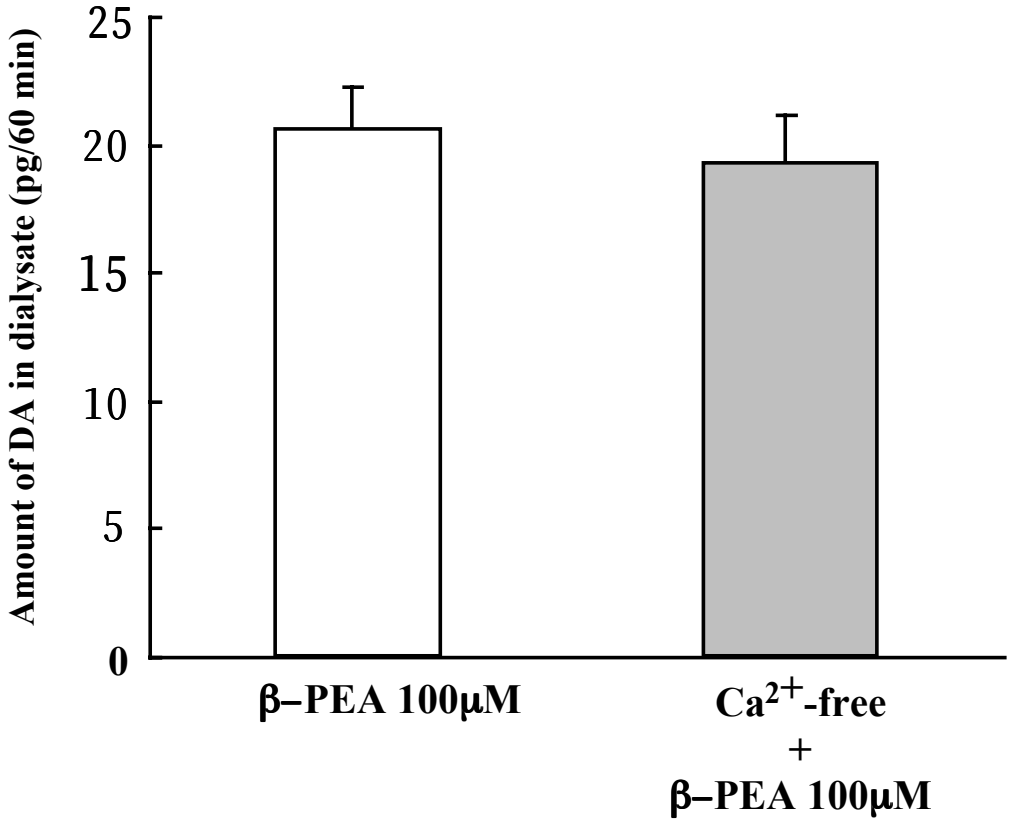

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**Fig. 7**