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Efffects of Fluoxetine on ATP-induced Calcium Signaling in PC12 Cells

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Fluoxetine, a widely used anti-depressant compound, has several additional effects, including blockade of voltage-gated ion channels. We examined whether fluoxetine affects ATP-induced calcium signaling in PC12 cells by using fura-2-based digital calcium imaging and assay for [^{3}H]-inositol phosphates (IPs). Treatment with ATP (100 μM) for 2 min induced $[Ca^{2+}]_i$ increases. The ATP-induced $[Ca^{2+}]_i$ increases were significantly decreased by removal of extracellular Ca^{2+} and treatment with the inhibitor of endoplasmic reticulum Ca^{2+} ATPase thapsigargin (1 μM). Treatment with fluoxetine for 5 min blocked the ATP-induced $[Ca^{2+}]_i$ increase concentration-dependently. Treatment with fluoxetine (30 μM) for 5 min blocked the ATP-induced $[Ca^{2+}]_i$ increase following removal of extracellular Ca^{2+} and depletion of intracellular Ca^{2+} stores. While treatment with the L-type Ca^{2+} channel antagonist nimodipine for 10 min inhibited the ATP-induced $[Ca^{2+}]_i$ increases significantly, treatment with fluoxetine alone blocked the ATP-induced responses. Treatment with fluoxetine also inhibited the 50 mM K^+-induced $[Ca^{2+}]_i$ increases completely. However, treatment with fluoxetine did not inhibit the ATP-induced [^{3}H]-IPs formation. Collectively, we conclude that fluoxetine inhibits ATP-induced $[Ca^{2+}]_i$ increases in PC12 cells by inhibiting both an influx of extracellular Ca^{2+} and a release of Ca^{2+} from intracellular stores without affecting IPs formation.

Key Words: ATP, Fluoxetine, Inositol phosphates, Non-selective cation channels, PC12 cells, Voltage-gated Ca^{2+} channels

INTRODUCTION

Fluoxetine is a widely used anti-depressant compound, its action is primarily attributed to inhibition of reuptake of serotonin into the synaptic cleft of central nervous system (Wong et al, 1995). Fluoxetine has additional blocking effects on voltage-gated ion channels (Rae et al, 1995; Farrugia, 1996; Tytgat et al, 1997; Pancrazio et al, 1998; Choi et al, 1999; Hahn et al, 1999; Deak et al, 2000; Pacher et al, 2000; Wang et al, 2003). Moreover, fluoxetine inhibits ligand-gated ion channels such as 5-hydroxytryptamine type 3 (5-HT_3)-receptor-gated channel (Fan, 1994a, b; Choi et al, 2003) and nicotinic acetylcholine receptor-gated channel (Garcia-Colunga et al, 1997).

Rat pheochromocytoma (PC12) cells have been reported to express $P2X_2$ (Brake et al, 1994) and P2Y purinoceptors (Unterberger et al, 2002). Adenosine triphosphate (ATP) induces increase in intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) in PC12 cells through activation of P2X receptor-operated non-selective cation channels (Fasolato et al, 1990; Nakazawa et al, 1990; Reber et al, 1992) and P2Y receptor-induced activation of phospholipase C (PLC) (Murrin & Boarder, 1992; Park et al, 1997). Moreover, the ATP-induced activation of non-selective cation channels depolarizes cell membrane, which increases $[Ca^{2+}]_i$ through activation of voltage-gated L-type Ca^{2+} channels (Hur et al, 2001). The ATP-induced $[Ca^{2+}]_i$ increases may be involved in the release of catecholamine in PC12 cells (Majid et al, 1992; Nakazawa & Inoue, 1992; Suh et al, 1995). However, fluoxetine has not been shown to affect ATP-induced calcium signaling via P2X receptors and/or P2Y receptors in neuronal cells.

We examined effects of fluoxetine on ATP-induced calcium signaling in PC12 cells by using fura-2-based digital calcium imaging and assay for [^{3}H]-inositol phosphates (IPs). We found that fluoxetine inhibited ATP-induced $[Ca^{2+}]_i$ increases in PC12 cells by inhibiting both an influx of extracellular Ca^{2+} and a release of Ca^{2+} from intracellular stores without affecting IPs formation.

METHODS

Materials

Fura-2 acetoxymethyl ester (AM) was purchased from

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ABBREVIATIONS: ATP, adenosine triphosphate; $[Ca^{2+}]_i$, intracellular Ca^{2+} concentration; 5-HT_3, 5-hydroxytryptamine type 3; IPs, inositol phosphates; IP_3, inositol 1,4,5-triphosphate; PC12, rat pheochromocytoma; PLC, phospholipase C.

Molecular Probes (Eugene, Ore., USA), Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS, heat-inactivated) from Invitrogen (Carlsbad, Calif., USA), and ATP disodium salt and all other reagents from Sigma (St. Louis, Mo, USA).

Cell culture

The PC12 cells were grown in DMEM with 10% fetal bovine serum, 100 μg/ml streptomycin, and 100 IU/ml penicillin at 37°C in a humidified atmosphere of 95% air and 5% CO_2. To measure $[Ca^{2+}]_i$, cells from the stock culture were plated onto glass coverslips (25 mm round) at a density of 3×10^4 cells/coverslip, and were used for experiments 2~3 days after plating.

Digital calcium imaging

Digital calcium imaging was performed as described by Rhie et al. (2003). The cells were loaded with 2 μM fura-2 AM in HEPES-buffered Hank's salts (HBSS) solution containing 0.5% bovine serum albumin for 45 min at 37°C. The HBSS solution was composed of the following (in mM): HEPES, 20; NaCl, 137; $CaCl_2$, 1.26; $MgSO_4$, 0.4; $MgCl_2$, 0.5; KCl, 5.0; KH_2PO_4, 0.4; Na_2HPO_4, 0.6; $NaHCO_3$, 3.0; and glucose, 5.0. To induce depolarization-induced activation of voltage-gated Ca^{2+} channels, we used 50 mM K^+ HBSS solution, in which 137.0 mM NaCl and 5.0 mM KCl were replaced with 92.3 mM NaCl and 50 mM KCl, respectively. The loading was terminated by washing with HBSS solution for 15 min prior to starting an experiment. The cover glass was then mounted in a flow through chamber (Thayer et al, 1988) which was superfused at 2 ml/min. Solutions were selected with a multi-port valve coupled to several reservoirs. The chamber containing the fura-2-loaded cells was mounted on the stage of an inverted microscope (Nikon TE300, Tokyo, Japan) and excited alternately at 340 and 380 nm by rapidly switching filters (10 nm band-pass) mounted on a computer-controlled wheel (Lambda 10-2, Sutter Instruments, Novato, Calif., USA) placed between a 100 W Xe arc lamp and the epifluorescence port of the microscope. Excitation light was reflected from a dichroic mirror (400 nm) through a 20× objective (Nikon; N.A. 0.5). Digital fluorescence images (510 nm, 40 nm band-pass) were collected with a cooled CCD camera (Photometrics; 1280×1035 binned to 256×207 pixels). Image pairs were collected every 6~20 seconds using an Axon Imaging Work Bench 2.2 (Axon Instruments, Foster City, Calif., USA); exposure to excitation light was 120 ms per image. Ratio was calculated from the two background-subtracted digital images.

Assay for [^{3}H]-inositol phosphates

After cells were grown in DMEM with 10% FBS for 2~3 days, cells were labeled with 10 ml of DMEM containing [^{3}H] myo-inositol (2 μCi/ml). At 24 h after labeling, cells were rinsed with 10 ml of HBSS solution containing 20 mM LiCl followed by incubation at 37°C for 30 min in 10 ml of the assay medium with the indicated drugs. Fluoxetine was added 20 min prior to the addition of ATP. The reaction was terminated by adding 0.4 ml of ice-cold 10% perchloric acid and incubated on ice for 30 min. After centrifugation, the supernatant was applied to Dowex AG 1×8 ion exchange column (100~200 mesh, formate form), total IPs were eluted with 2.5 ml of 1.5 M ammonium formate in 0.1 N formic acid, and radioactivity was determined by liquid scintillation counting. IP formation was estimated by determining the ratio of [^{3}H]-IPs to [^{3}H]-inositol plus [^{3}H]-IPs (Rhie et al, 2003).

Data analysis

Data are expressed as means±SEM. The significance of differences between means was determined with Student's *t*-test or one-way ANOVA followed by a Bonferroni's test. The IC_{50} of fluoxetine was calculated using a non-linear least-squares fit of the Hill equation to the concentration/response data (Origin 7.0; Microcal Software, Northampton, Mass., USA).

RESULTS

Inhibitory effects of fluoxetine on ATP-induced $[Ca^{2+}]_i$ increases in PC12 cells

Treatment with ATP (100 μM) for 2 min induced $[Ca^{2+}]_i$ transients in PC12 cells, with peaks between 48 and 80 s, 25 s after the treatment. The increased $[Ca^{2+}]_i$ levels returned to near basal levels between 3.9 and 6.5 min. Reproducible responses could be elicited by applying ATP (100 μM) for 2 min at 30 min intervals (peak 2/peak 1= 1.02±0.01, n=98) (Figs. 1A and C). In order to determine whether fluoxetine affects the ATP-induced $[Ca^{2+}]_i$ increases, we pretreated the cells with various concentrations of fluoxetine (10 nM, 100 nM, 1 μM, 10 μM, 30 μM) for 5 min. The treatment with fluoxetine (10 nM) for 5 min did not significantly affect the ATP-induced $[Ca^{2+}]_i$ increases (peak 2/peak 1=1.05±0.06, n=30), whereas the treatment with increasing concentrations of fluoxetine (100 nM→30 μM) significantly inhibited the ATP-induced responses in concentration-dependent manner (peak 2/peak 1=0.89± 0.01 in 100 nM, 0.61±0.01 in 1 μM, 0.12±0.01 in 10 μM, 0.01±0.00 in 30 μM) (Fig. 1). A non-linear, least squares fit of Hill equation yielded an IC_{50} for fluoxetine of 1.25± 0.59 μM and a Hill coefficient of 1.17±0.33 (Fig. 1C).

Inhibitory effects of fluoxetine on the ATP-induced Ca^{2+} release from intracellular stores and Ca^{2+} influx from extracellular space

In PC12 cells, ATP induces Ca^{2+} influx from extracellular space and/or Ca^{2+} release from intracellular stores. We tried to determine which mechanisms are involved in the inhibition of fluoxetine on the ATP-induced $[Ca^{2+}]_i$ increases. We observed whether removal of extracellular Ca^{2+} by treatment with nominally Ca^{2+}-free HBSS solution or depletion of intracellular Ca^{2+} stores by treatment with the inhibitor of endoplasmic reticulum Ca^{2+} ATPase thapsigargin (Thastrup et al, 1990) affects the ATP-induced $[Ca^{2+}]_i$ increases in both the absence and the presence of fluoxetine. Reproducible responses could be elicited by applying ATP (100 μM) for 2 min at 30 min intervals (peak 2/peak 1=0.96±0.01, n=19). Removal of Ca^{2+} for 2 min markedly inhibited the subsequent ATP-induced $[Ca^{2+}]_i$ increases, but ATP still induced the small $[Ca^{2+}]_i$ increases (peak 2/peak 1=0.28±0.02, n=31) (Figs. 2B and D). Pretreatment with both fluoxetine for 5 min and nominally Ca^{2+}-free HBSS solution for 2 min blocked the ATP-induced re-

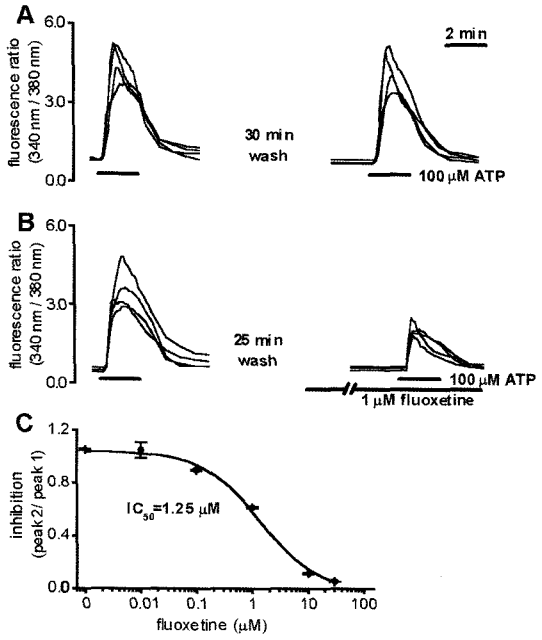

Fig. 1. Concentration-dependent inhibitory effects of fluoxetine on ATP-induced $[Ca^{2+}]_i$ increases in PC12 cells. (A) Reproducible $[Ca^{2+}]_i$ increases were elicited by superfusion with 100 μM ATP for 2 min at 30 min intervals. (B) After cells were pretreated with fluoxetine (1 μM) for 5 min, the subsequent ATP-induced $[Ca^{2+}]_i$ responses were observed. Image pairs were collected at 6~20 s intervals. ATP and fluoxetine were applied as indicated by the horizontal bars. (C) Concentration/response data for effects of fluoxetine on the ATP-induced $[Ca^{2+}]_i$ increases. The ATP-induced response amplitude is presented as a ratio of the initial control (peak 2/peak 1) (n=21, 30, 29, 15, 21, 29 at 0, 10 nM, 100 nM, 1 μM, 10 μM, 30 μM). A nonlinear least-squares fit of the Hill equation (Hill coefficient= 1.17±0.33) to the concentration/response data yielded an IC_{50} of 1.25±0.59 mM for fluoxetine. Data are means±SEM.

sponses completely (n=21). Treatment with thapsigargin (1 μM) for 15 min decreased the subsequent ATP-induced responses significantly (peak 2/peak 1=0.61±0.01, n=33). Pretreatment with fluoxetine for 5 min blocked the ATP-induced responses in the thapsigargin-treated cells completely (n=22) (Figs. 2C and D).

Inhibitory effects of fluoxetine on the ATP-induced secondary Ca^{2+} influx through voltage-gated Ca^{2+} channels

ATP depolarizes the membrane through P2X receptor-mediated influx of Ca^{2+} and Na^+, which secondarily activates voltage-gated L-type Ca^{2+} channels in PC12 cells (Hur et al, 2001). It has also been reported that fluoxetine inhibits the voltage-gated Ca^{2+} channels in PC12 cells (Hahn et al, 1999). We tested whether fluoxetine affects the ATP-induced secondary activation of voltage-gated L-type Ca^{2+} channels. Pretreatment with nimodipine (1 μM) for 10 min inhibited the subsequent ATP-induced $[Ca^{2+}]_i$ increases significantly (peak 2/peak 1=0.47±0.01, n=12). Following washout of the nimodipine for 25 min, treatment with fluoxetine (30 μM) for 5 min blocked the ATP-induced responses (n=31) (Figs. 3B and C). To confirm the inhibitory effects of fluoxetine on the Ca^{2+} influx through ATP-induced activation of voltage-gated Ca^{2+} channels, we determined whether fluoxetine inhibits the depolarization-induced activation of Ca^{2+} channels by 50 mM K^+ HBSS solution. Reproducible Ca^{2+} increases were induced by treatment with 50 mM K^+ HBSS solution for 90 s at 30 min intervals (peak 2/peak 1=0.92±0.01, n= 23). Treatment with fluoxetine (30 μM) for 5 min inhibited the subsequent high K^+-induced $[Ca^{2+}]_i$ responses completely (peak 2/peak 1=0.01±0.00, n=53).

Effects of fluoxetine on the ATP-induced [^{3}H]-IPs formation

ATP can activate inositol 1,4,5-triphosphate (IP_3)-induced Ca^{2+} release from intracellular stores by PLC activation in PC12 cells (Murrin & Boarder, 1992; Park et al, 1997). In Fig. 2, ATP still induced the $[Ca^{2+}]_i$ increases following removal of extracellular Ca^{2+}. Treatment with fluoxetine completely blocked the ATP- induced responses following removal of extracellular Ca^{2+}. These results suggest that fluoxetine inhibits release of Ca^{2+} from intracellular stores in PC12 cells. In the next experiment, we determined whether fluoxetine inhibits a release of Ca^{2+} from intracellular stores through an inhibition of ATP-induced phosphoinositide hydrolysis or an inhibition of IP_3-induced Ca^{2+} release without an inhibition of the phosphoinositide hydrolysis. We measured the ATP- induced [^{3}H]-IPs formation, which results from PLC activation, in the presence or the absence of fluoxetine. Treatment with 100 μM ATP for 30 min significantly increased [^{3}H]-IPs formation (4.3±0.2% of [^{3}H]-inositol plus [^{3}H]-IPs in control, 8.3±0.8% of [^{3}H]-inositol plus [^{3}H]-IPs in 100 μM ATP, n=4, $P<0.05$). The ATP-induced [^{3}H]-IPs formation was not inhibited by treatment with 30 μM fluoxetine for 30 min (5.1±0.2% of [^{3}H]-inositol plus [^{3}H]- IPs in fluoxetine, 9.9± 0.8% of [^{3}H]-inositol plus [^{3}H]-IPs in 100 μM ATP+ fluoxetine, n=4).

DISCUSSION

The present study clearly shows that fluoxetine inhibits the ATP-induced $[Ca^{2+}]_i$ increases in PC12 cells. The inhibitory effect of fluoxetine on the ATP-induced $[Ca^{2+}]_i$ increases was mediated by blocking both the ATP-induced Ca^{2+} influx from extracellular space and the ATP-induced release of Ca^{2+} from intracellular stores. However, fluoxetine did not inhibit the ATP-induced PLC activation.

ATP has been reported to induce $[Ca^{2+}]_i$ increase in PC12 cells by an influx of non-selective cations such as Ca^{2+} and Na^{2+} (Fasolato et al, 1990; Nakazawa et al, 1990; Reber et al, 1992; Choi & Kim, 1996; Park et al, 1997). In this study, removal of extracellular Ca^{2+} markedly inhibited the ATP-induced $[Ca^{2+}]_i$ increases. Treatment with fluoxetine inhibited the ATP-induced $[Ca^{2+}]_i$ responses following depletion of intracellular Ca^{2+} stores with thapsigargin. Furthermore, treatment with nimodipine partially inhibited the ATP-induced $[Ca^{2+}]_i$ increases, while treatment with fluoxetine blocked the ATP-induced $[Ca^{2+}]_i$ responses. These data suggest that fluoxetine inhibits the ATP-induced Ca^{2+} influx by inhibiting non-selective cation channels. It is indirectly supported by earlier studies that fluoxetine inhibits ligand-gated ion channels such as 5-HT_3-receptor-gated channel (Fan, 1994a, b; Choi et al, 2003) and nicotinic acetylcholine receptor-gated channel

(Garcia-Colunga et al, 1997).

Fluoxetine has been reported to inhibit the voltage-gated Ca^{2+} channels in rat spinal cord and cortical synaptosomes (Stauderman et al, 1992; Wang et al, 2003) and in PC12 cells (Hahn et al, 1999). Fluoxetine also inhibits three types of voltage-gated Ca^{2+} channels (T-type, N-type and L-type)

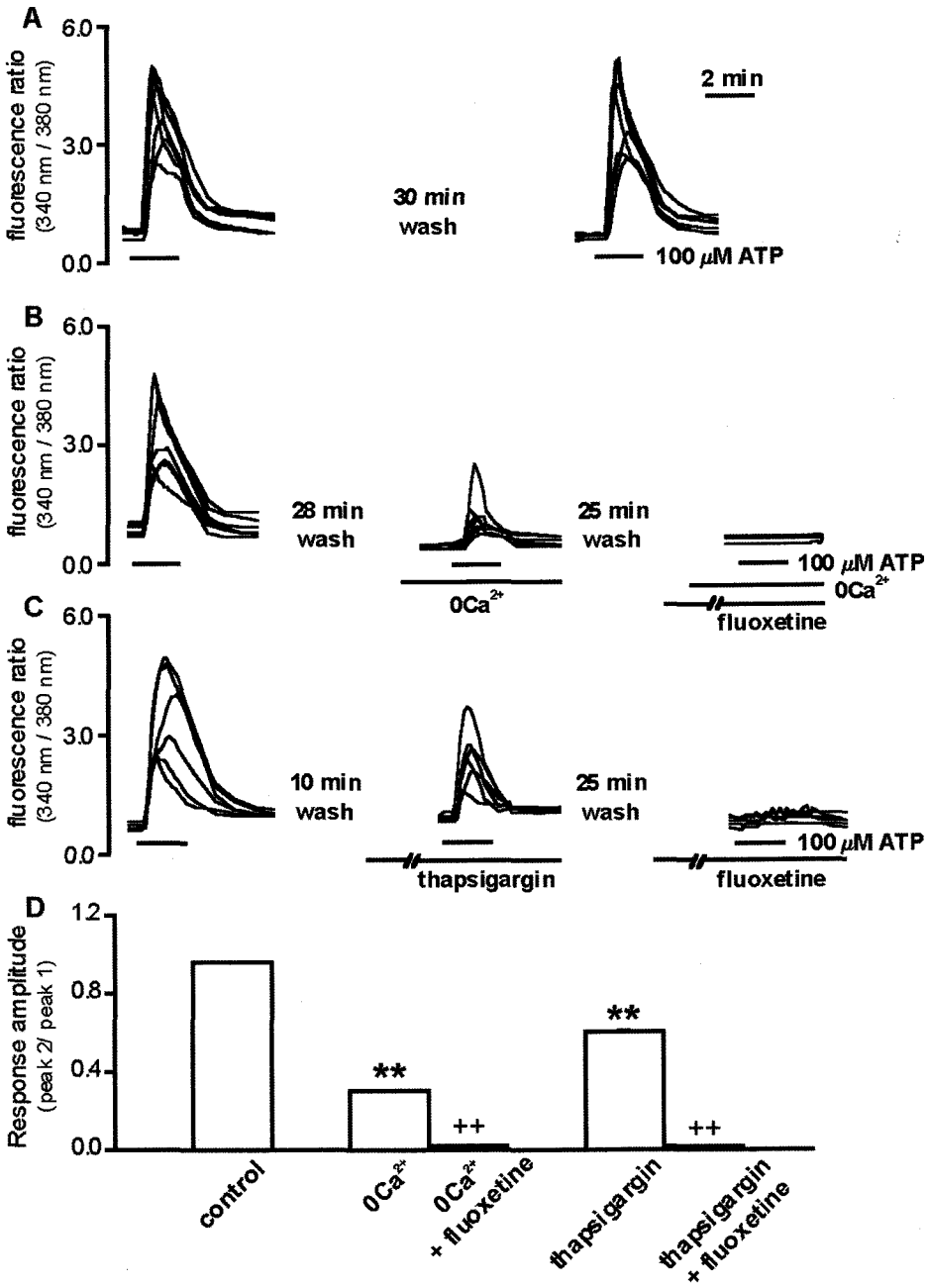

Fig. 2. Inhibitory effects of fluoxetine on ATP-induced Ca^{2+} release from intracellular stores and Ca^{2+} influx from extracellular space. A, Reproducible $[Ca^{2+}]_i$ increases were elicited by superfusion with 100 μM ATP for 2 min at 30 min intervals. After the ATP-induced $[Ca^{2+}]_i$ increases were observed following each treatment with nominally Ca^{2+} -free HBSS solution for 2 min (B) and thapsigargin (1 μM) for 15 min (C), the ATP-induced responses were observed in the presence of fluoxetine (30 μM) for 5 min. (D) Summary of effects of fluoxetine on the ATP-induced $[Ca^{2+}]_i$ increases. The ATP-induced response amplitude is presented as a ratio of the initial control (peak 2/peak 1) for control (n=19), nominally Ca^{2+}-free HBSS solution-treated (0 Ca^{2+}, n=31), 0 Ca^{2+} and fluoxetine- treated (n=21), thapsigargin-treated (n=33), and thapsigargin plus fluoxetinetreated (n=22) cells. Data are means± SEM. **$p<0.01$ relative to control, $^{++}p<0.01$ relative to respective non-fluoxetine- treated cells (one-way ANOVA followed by Bonferroni's test).

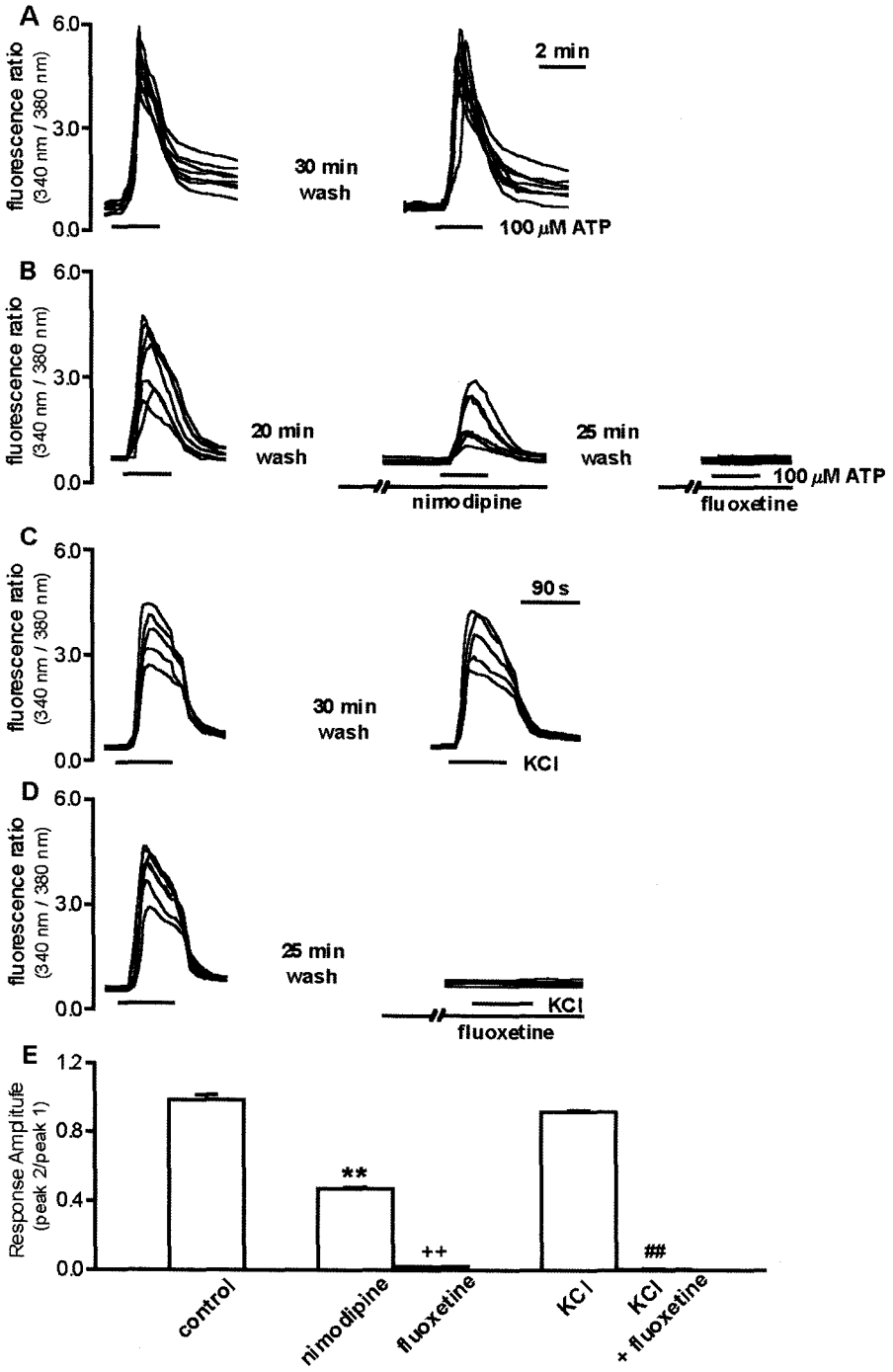

Fig. 3. Inhibitory effects of fluoxetine on the ATP-induced secondary Ca^{2+} influx through voltage-gated Ca^{2+} channels. A, Reproducible $[Ca^{2+}]_i$ increases were elicited by superfusion with 100 μM ATP for 2 min at 30 min intervals. B, ATP-induced $[Ca^{2+}]_i$ increases were induced in the presence of the L-type Ca^{2+} channel antagonist nimodipine (1 μM) for 10 min following a 2 min exposure to ATP and 20 min wash. ATP was applied for 2 min following exposure to fluoxetine for 5 min. C, $[Ca^{2+}]_i$ increases induced by superfusion with 50 mM KCl HBSS solution for 90 s at 30 min intervals in the absence (C) and presence (D) of fluoxetine (30 μM) for 5 min. E, Summary of effects of fluoxetine and nimodipine on the ATP- or high K^+-induced $[Ca^{2+}]_i$ increases. The ATP-induced response amplitude is presented as a ratio of the initial control (peak 2/peak 1) for control (n=47), nimodipine-treated (n=12), nimodipine plus fluoxetine-treated (n=45), KCl-treated (n=23), and KCl plus fluoxetinetreated (n=53) cells. Data are means SEM. ** $P<0.01$ relative to control, $^{++}P<0.01$ relative to respective non-fluoxetine-treated cells, $^{\#\#}P<0.01$ relative to KCl-treated cells (one-way ANOVA followed by Bonferroni's test).

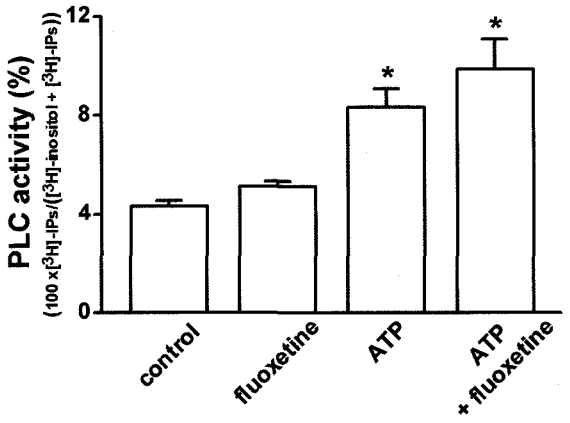

Fig. 4. Effects of fluoxetine on ATP-induced [^{3}H]-inositol phosphates (IPs) formation. Cells were assayed for their responses to vehicle (control), fluoxetine (30 μM), ATP (100 μM), and fluoxetine (30 μM) plus ATP (100 μM). PLC activity was estimated by determining the percentage of [^{3}H]-IPs relative to [^{3}H]-inositol plus [^{3}H]-IPs. Data are expressed as means ± SEM of four separate experiments. *$P < 0.05$ relative to control (one-way ANOVA followed by Bonferroni's test).

in hippocampal pyramidal neurons (Deak et al, 2000). In PC12 cells, ATP can induce Ca^{2+} influx through secondaryactivation of voltage-gated Ca^{2+} channels (mostly L-type) following depolarization of the membrane by ATP-induced activation of non-selective cation channels (Gollasch & Haller, 1995; Hur et al, 2001). In this study, while treatment with nimodipine inhibited the ATP-induced $[Ca^{2+}]_i$ increases to 0.47% of control responses, treatment with fluoxetine blocked the ATP-induced responses following the washout of nimodipine. Moreover, pretreatment with fluoxetine completely inhibited the depolarization-induced $[Ca^{2+}]_i$ increases by high K^+. All of these data suggest that fluoxetine inhibits ATP-induced $[Ca^{2+}]_i$ increases in PC12 cells through inhibition of secondary activation of voltage-gated L-type Ca^{2+} channels by ATP.

ATP induces $[Ca^{2+}]_i$ increases in PC12 cells through PLC activation (Park et al, 1997). In this study, ATP induced increase in [^{3}H]-IPs formation as well as $[Ca^{2+}]_i$ increase following removal of extracellular Ca^{2+}. Treatment with fluoxetine inhibited the ATP-induced $[Ca^{2+}]_i$ increases following removal of extracellular Ca^{2+}. However, treatment with fluoxetine did not affect the ATP-induced [^{3}H]-IPs formation. These data indicate that fluoxetine inhibits ATP-induced release of Ca^{2+} from IP_3-sensitive intracellular stores without inhibiting PLC activity. These data suggest that fluoxetine inhibits the ATP-induced release of Ca^{2+} from IP_3-sensitive intracellular stores. These data are supported by other's report that acute treatment with fluoxetine did not have any effects on the phosphoinositide metabolism in rat brain (Dwivedi et al, 2002).

In the present study, fluoxetine inhibits Ca^{2+} release from intracellular store without affecting PLC activity. The inhibitory effects of fluoxetine on the Ca^{2+} channels were not mediated by protein phosphorylation and/or G protein (Hahn et al, 1999). These results suggest that the effect of fluoxetine may be more specific to channels than other targets. However, fluoxetine blocks a wide variety of ligand- and voltage-gated ion channels. In addition, the inhibitory effects are not specific to certain type of ion channels. Moreover, the action site of fluoxetine on channels is still controversial (Garcia-Counga et al, 1997; Choi et al, 1999; Choi et al, 2003). Therefore, we speculate that fluoxetine nonspecifically inhibits on a variety of channels including ATP-induced Ca^{2+} channels.

The IC_{50} of fluoxetine in the inhibition of ATP-induced Ca^{2+} increases is 1.25 μM. Therapeutic plasma concentrations have been reported to be within 0.15~1.5 μM (Orsulak et al, 1988; Kelly et al, 1989). Under certain conditions, the plasma concentration of fluoxetine can reach higher levels (Pato et al, 1991; Borys et al, 1992; Karson et al, 1993; Komoroski et al, 1994). In addition, fluoxetine can be accumulated in human brain (Karson et al, 1993; Komoroski et al, 1994), suggesting that a significant reduction of the ATP-induced activation of various Ca^{2+} channels by fluoxetine may occur in patients chronically treated with fluoxetine.

ATP is an important transmitter in both central nervous system and peripheral nervous system (Ralevic & Burnstock, 1998). ATP-induced $[Ca^{2+}]_i$ increases have been reported to be involved in release of cathecholamine in PC12 cells (Majid et al, 1992; Nakazawa & Inoue, 1992; Suh et al, 1995). Furthermore, there are evidences suggesting that ATP regulates various functions including release of neurotransmitters in the peripheral and central nervous system (Cunha & Ribeiro, 2000). Although fluoxetine is widely used as an anti-depressant drug, fluoxetine can also affect various neural functions in the peripheral nervous system and the central nervous system through inhibition of ATP-induced Ca^{2+} influx, secondary activation of voltage-gated Ca^{2+} channels, and Ca^{2+} release from IP_3-sensitive intracellular stores in neuronal cells.

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