RESEARCH ARTICLE

Stimulatory effects of the soy phytoestrogen genistein on noradrenaline transporter and serotonin transporter activity

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Received: April 9, 2009
Revised: September 19, 2009
Accepted: September 24, 2009

We examined the effects of genistein, one of the major soy phytoestrogens, on the activity of noradrenaline transporter (NAT) and serotonin transporter. Treatment with genistein (10 nM–10 μM) for 20 min stimulated [^{3}H]noradrenaline (NA) uptake by SK-N-SH cells. Genistein also stimulated [^{3}H]NA uptake and [^{3}H]serotonin uptake by NAT and serotonin transporter transiently transfected COS-7 cells, respectively. Kinetics analysis of the effect of genistein on NAT activity in NAT-transfected COS-7 cells revealed that genistein significantly increased the maximal velocity of NA transport with little or no change in the affinity. Scatchard analysis of [^{3}H]nisoxetine binding to NAT-transfected COS-7 cells showed that genistein increased the maximal binding without altering the dissociation constant. Although genistein is also known to be an inhibitor of tyrosine kinases, daidzein, another soy phytoestrogen and an inactive genistein analogue against tyrosine kinases, had little effect on [^{3}H]NA uptake by SK-N-SH cells. The stimulatory effects on NAT activity were observed by treatment of tyrphostin 25, an inhibitor of epidermal growth factor receptor tyrosine kinase, whereas orthovanadate, a protein tyrosine phosphatase inhibitor, suppressed [^{3}H]NA uptake by NAT-transfected COS-7 cells. These findings suggest that genistein up-regulates the activity of neuronal monoamine transporters probably through processes involving protein tyrosine phosphorylation.

Keywords:
Genistein / Noradrenaline transporter / Serotonin transporter / Tyrosine kinase inhibition / Up-regulation

1 Introduction

Genistein, an isoflavone, is a major natural phytoestrogen found in soybeans. Considerable research attention has been focused on the high dietary intake of soy isoflavones because of their potentially beneficial effects associated with reduced risks of developing osteoporosis [1], high cholesterol [2], menopausal symptoms [3], and some forms of cancer [4]. The structure of genistein is similar to the primary structure of 17β-estradiol [5], which leads to its weak estrogenic agonist or antagonist activity that influences cell functions mediated through

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Abbreviations: B_{max}, maximal binding; **bNAT,** bovine noradrenaline transporter; K_d, dissociation constant; K_m, Michaelis–Menten constant; **KRH,** Krebs–Ringer HEPES; **NA,** noradrenaline; **NAT,** noradrenaline transporter; **PP2,** 4-amino-5-(4-chlorophenyl)-7-(*t*-butyl)pyrazole[3,4-*d*]pyrimidine; **rSERT,** rat serotonin transporter; **SERT,** serotonin transporter; V_{max}, maximal velocity; **5-HT,** serotonin

cytosolic/nuclear estrogen receptors (ER-α and ER-β) in genomic processes [5].

In addition to these genomic actions, evidence has grown that phytoestrogens and estrogens have an effect on a variety of cell functions by nongenomic mechanisms [6, 7]. For instance, estrogens rapidly modulate the neural functions of membrane proteins such as neurotransmitter receptors [8], transporters [9], and ion channels [10]. Genistein has effects that are independent of its estrogenic activity, including protein tyrosine kinase inhibition, immune system modulation, and antioxidant activity [11]. In particular, genistein is a well-known general inhibitor of tyrosine kinases that play important roles in diverse cellular signaling pathways, including various neuronal transmissions [12]. Previous studies have reported that several types of ion channels and transporters are functionally modified by tyrosine phosphorylation [13, 14].

Biogenic monoamines, such as noradrenaline (NA) and serotonin (5-HT), are neurotransmitters in the central and peripheral nervous systems. A key regulatory process of chemical neurotransmission is the inactivation of neurotransmitters after their release, mainly through the action of biogenic monoamine transporters. The neuronal transfer and synaptic clearance of neurotransmitters are controlled by plasma membrane monoamine transporters such as noradrenaline transporter (NAT) and serotonin transporter (SERT) in the presynaptic nerve terminal. Therefore, NAT or SERT mediate the termination of neurotransmission by the reuptake of NA or 5-HT released into the extracellular medium, respectively [15, 16]. NAT and SERT also are known to be the cellular targets for tricyclic antidepressants and pyschostimulants such as cocaine and amphetamine [17, 18].

Previously, we reported that bisphenol A, an environmental estrogenic pollutant, as well as 17β-estradiol inhibit NAT activity in cultured bovine adrenal medullary cells [19]. Furthermore, several reports have shown that genistein decreased NAT activity at high concentrations [20] and estradiol and glabridin, phytoestrogen, inhibited 5-HT uptake [21]. These results prompted us to investigate the effects of phytoestrogens on NAT function at physiologically relevant concentration. In the present study, we examined the acute effects of genistein, a soy phytoestrogen, on the function of NAT or SERT in noradrenergic neuroblastoma SK-N-SH cells and COS-7 cells transiently transfected with NAT or SERT at physiologically relevant concentration. Unexpectedly, we found that genistein, but not daidzein, increased both NAT and SERT activities probably *via* inhibition of tyrosine kinase activity in the cells.

2 Materials and methods

2.1 Materials

Reagents were obtained from the following sources: α-minimum essential medium, DMEM, penicillin-streptomycin, Gibco/Invitrogen Carlsbad, CA, USA; fetal bovine serum, JRH Bioscience, Lenexa, KS, USA; genistein, sodium orthovanadate, pargyline hydrochloride and *l*-ascorbic acid, Nacalai Tesque, Kyoto, Japan; daidzein, coumestrol, *l*-NA, 5-HT, desipramine hydrochloride, and ICI 182 780, Sigma, St. Louis, MO, USA; nisoxetine hydrochloride, Research Biochemicals International (RBI), Natick, MA, USA; 4-amino-5-(4-chlorophenyl)-7-(*t*-butyl)-pyrazole[3,4-*d*]pyrimidine (PP2), tyrphostin 25, Calbiochem, La Jolla, CA, USA; *l*-[7,8-^{3}H]-noradrenaline (1.55 TBq/mmol), GE Healthcare, UK; hydroxytryptamine creatinine sulfate, 5-[1,2-3H(N)] (791.8 GBq/mmol), nisoxetine hydrochloride [N-methyl-^{3}H] (2960 GBq/mmol), Perkin-Elmer Life Sciences, Boston, MA, USA.

2.2 Cell culture and transfection

The human neuroblastoma cell line, SK-N-SH (RCB0426), and COS-7 (RCB0539) cells were provided by the RIKEN Cell Bank (Tsukuba, Japan). SK-N-SH cells were maintained in culture medium containing α-minimum essential medium supplemented with 10% fetal bovine serum, 100 units/mL penicillin, and 100 μg/mL streptomycin. Cells were plated on poly-L-lysine coated plates at a density of 1×10^6 cells *per* well in 5% CO_2/95% air for each experiment. COS-7 cells were maintained in culture medium containing DMEM supplemented with 10% fetal bovine serum, 100 units/mL penicillin, and 100 μg/mL streptomycin. Bovine noradrenaline transporter (bNAT) cDNA was kindly provided by Dr. H. Bönisch (University of Bonn, Bonn, Germany) and rat serotonin transporter (rSERT) cDNA was used as previously reported [22]. Transfection of bNAT and rSERT cDNA was performed using Effectene Transfection Reagent (Qiagen, Hilden, Germany) at 10:1 reagent:cDNA ratios. Cells were incubated after transfection for 24–48 h before use in experiments.

2.3 [^{3}H]NA and [^{3}H]5-HT uptake by cells

Cells (1×10^6/well) were preincubated at 37°C for various periods in Krebs–Ringer HEPES (KRH) buffer (composition: 120 mM NaCl, 4.7 mM KCl, 1.2 mM $MgCl_2$, 2.2 mM $CaCl_2$, 1.2 mM KH_2PO_4, 25 mM HEPES, pH 7.4, and 10 mM glucose) in the presence or absence of test compounds. The cells were further incubated with KRH buffer containing 10 μM pargyline, 100 μM ascorbic acid, and [^{3}H]NA (0.1 μM, 0.1 μCi) or [^{3}H]5-HT (50 nM, 0.1 μCi) at 37°C for 10 min in the presence or absence of test compounds. To determine the saturation kinetic of [^{3}H]NA uptake, cells were incubated with various concentrations (1–10 μM) of [^{3}H]NA in the presence or absence of 10 μM genistein. After incubation, the cells were rapidly washed four times with 1 mL of ice-cold KRH buffer and solubilized in 1 mL of 10% TritonX-100. The radioactivity in the solubilized cells was counted with a liquid

scintillation counter (Tri-Carb 2900TR; Packard BioScience, Meriden, CT., USA). Nonspecific uptake was determined in the presence of 10 μM desipramine (for [^{3}H]NA uptake) or 50 μM imipramine (for [^{3}H]5-HT uptake) and specific uptake was obtained by subtracting the nonspecific uptake from the total uptake.

2.4 [^{3}H]Nisoxetine binding to cells

According to a previously reported method for the equilibrium binding of [^{3}H]nisoxetine to the intact cells [23], cells were incubated with various concentrations of [^{3}H]nisoxetine in a binding buffer at 4°C for 2 h in the presence or absence of 10 μM genistein. After incubation, cells were washed with ice-cold binding buffer (100 mM NaCl, 50 mM Tris-HCl, pH 8.0, and 100 mM ascorbic acid). Specific binding of [^{3}H]nisoxetine was defined as the total binding minus the nonspecific binding, which was determined in the presence of 10 μM nisoxetine.

2.5 Statistics

Results are expressed as means ± SEM of multiple determinations. Statistical analysis was carried out by one-way analysis of variance with *post hoc* mean comparison using the Fisher's protected least significant difference (PLSD). When $p < 0.05$ differences were considered to be statistically significant.

3 Results

3.1 Effects of phytoestrogens on [^{3}H]NA uptake by SK-N-SH cells

We examined the effects of phytoestrogens, such as genistein, daidzein, and coumestrol, on [^{3}H]NA uptake by SK-N-SH cells. The cells were pretreated with or without genistein at 10 μM for the indicated periods (0–30 min), and then were incubated for another 10 min with [^{3}H]NA in the presence or absence of genistein (10 μM). Genistein caused a rapid increase in [^{3}H]NA uptake during the first 10 min and subsequently a gradual increase at 10–30 min (Fig. 1A). Pretreatment of cells with 0.1–10 μM genistein for 20 min caused a significant increase in [^{3}H]NA uptake (Fig. 1B). As shown in Fig. 1B, treatment with daidzein and coumestrol, other phytoestrogens, had little effect at any concentration (0.01–100 μM).

3.2 Effects of genistein on [^{3}H]NA and [^{3}H]5-HT uptake by bNAT or rSERT transfected COS-7 cells

Treatment with genistein at 0.01–10 μM for 20 min stimulated [^{3}H]NA uptake by bNAT transiently transfected COS-7 cells (Fig. 2A). At a high concentration (100 μM), however, genistein slightly suppressed [^{3}H]NA uptake. This concentration-dependent stimulatory effect of genistein (0.1–10 μM) was also observed in rSERT transiently transfected COS-7 cells (Fig. 2B).

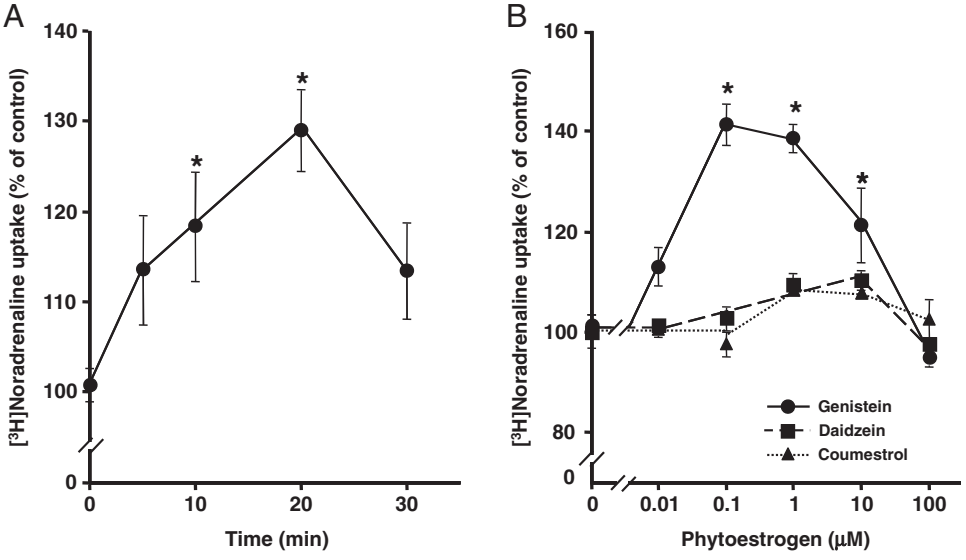

Figure 1. Effects of phytoestrogens on [^{3}H]NA uptake by SK-N-SH cells. (A) Cells were pretreated with or without genistein (10 μM) for the indicated periods (0–30 min) and then incubated for another 10 min with [^{3}H]NA (0.1 μM, 0.1 μCi) in the presence or absence of 10 μM genistein. The desipramine-sensitive [^{3}H]NA uptake by the cells was measured. (B) Cells were pretreated for 20 min with or without various concentrations (0.01–100 μM) of genistein, daidzein, and coumestrol, and then the desipramine-sensitive uptake of [^{3}H]NA by the cells was measured for 10 min in the presence or absence of various concentrations of genistein, daidzein, and coumestrol. Results are presented as percentage of control values (62.3 ± 4.2 fmol/1 × 10^6 cells/min). Data are means ± SEM from three separate experiments. *p<0.05, compared with control.

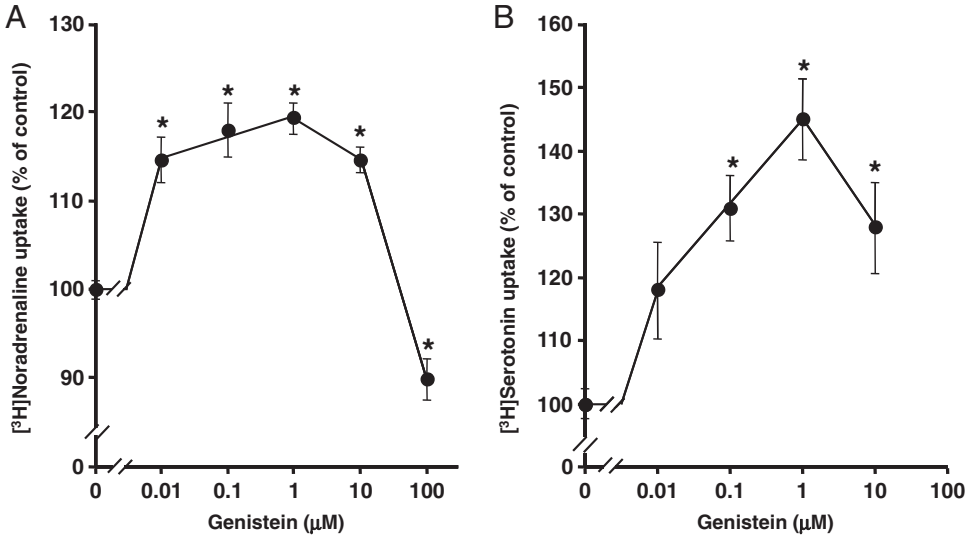

Figure 2. Effects of genistein on [^{3}H]NA and [^{3}H]5-HT uptake by bNAT (A) or rSERT (B) transfected COS-7 cells. (A) The bNAT transfected COS-7 cells were pretreated for 20 min with various concentrations of genistein (0.01–100 μM), and then the desipramine-sensitive uptake of [^{3}H]NA by the cells was measured. (B) The rSERT transfected COS-7 cells were pretreated with various concentrations of genistein (0.01–10 μM) for 20 min, and then incubated for another 10 min with [^{3}H]5-HT (50 nM, 0.1 μCi) in the presence of various concentrations of genistein. The imipramine-sensitive uptake of [^{3}H]5-HT was measured. Results are presented as percentage of control values (74.2 ± 6.6 fmol/1 × 10^6 cells/min; [^{3}H]NA uptake, 215 ± 16 fmol/1 × 10^6 cells/min; [^{3}H]5-HT uptake). Data are means ± SEM from three separate experiments. *$p < 0.05$, compared with control.

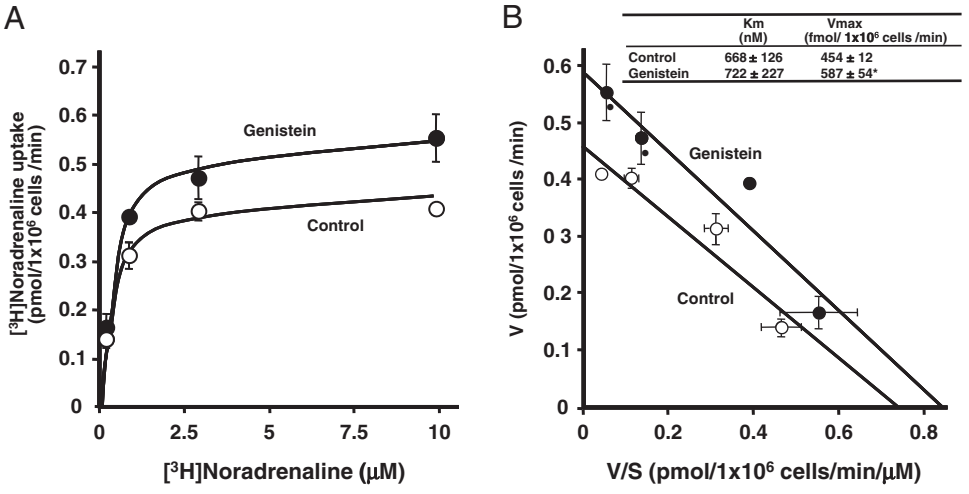

Figure 3. Saturation curves and Eadie–Hofstee plots of [^{3}H]NA uptake by bNAT transfected COS-7 cells. (A) Cells were pretreated with or without genistein (10 μM) for 20 min at 37°C. Desipramine-sensitive [^{3}H]NA uptake was measured with various concentrations (0.1–10 μM) of [^{3}H]NA. (B) Eadie–Hofstee analysis of [^{3}H]NA uptake obtained from (A). Data are means ± SEM from three separate experiments. Inset table: K_m and V_{max} values were calculated by Eadie–Hofstee analysis of the saturation curves in the absence of a drug (control) or in the presence of 10 μM genistein. K_m and V_{max} values measured from control cells were 668 ± 126 nM and 454 ± 12 fmol/1 × 10^6 cells/min, respectively, whereas those of genistein-treated cells were 722 ± 227 nM and 587 ± 54 fmol/1 × 10^6 cells/min, respectively. *$p < 0.05$, compared with control.

Incubation of bNAT transfected COS-7 cells showed a saturable [^{3}H]NE uptake with increasing concentrations of [^{3}H]NA (0.1–10 μM) (Fig. 3A). From the Eadie–Hofstee analysis (Fig. 3B), the Michaelis–Menten constant (K_m) value for [^{3}H]NA uptake was 668 ± 126 nM, and the maximal velocity (V_{max}) was 454 ± 12 fmol/1 × 10^6cells/min in control cells. Treatment of genistein at 10 μM produced a significant increase in V_{max} (587 ± 54 fmol/1 × 10^6cells/min) without altering K_m (722 ± 227 nM).

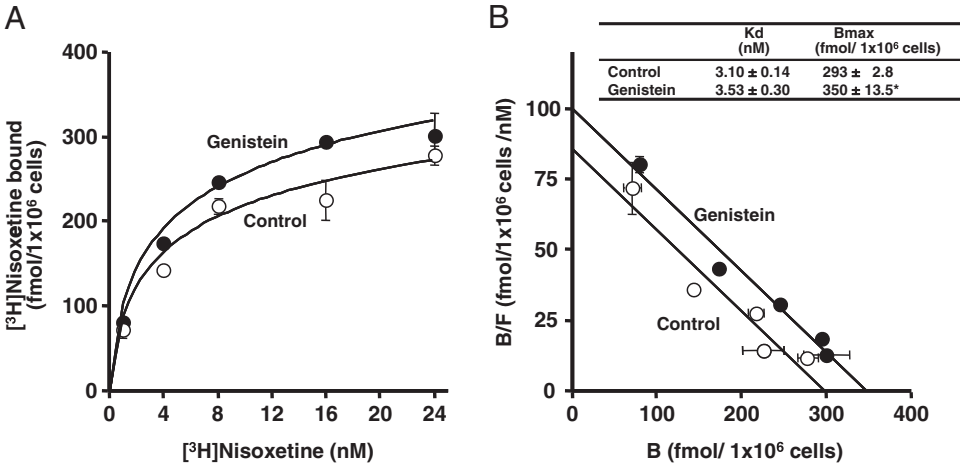

Figure 4. Saturation curves (A) and Scatchard plots (B) of specific [^{3}H]nisoxetine binding in intact bNAT transfected COS-7 cells. (A) The bNAT transfected COS-7 cells were incubated with increasing concentrations of [^{3}H]nisoxetine (1–24 nM) in the presence or absence of genistein (10 μM) at 4°C for 2 h. Non-specific binding was determined in the presence of 10 μM nisoxetine. The specific binding was 30–40% of the total binding at the K_d concentration of [^{3}H]nisoxetine. (B) The Scatchard plots are calculated from (A). Data are means ± SEM from three separate experiments. Inset table: K_d and B_{max} values were calculated by the Scatchard plot analysis in the absence of a drug (control) or in the presence of 10 μM genistein. K_d and B_{max} values measured from control cells were 3.10 ± 0.14 nM and 293 ± 3 fmol/1×10^6cells, respectively, whereas those of genistein-treated cells were 3.53 ± 0.30 nM and 350 ± 14 fmol/1×10^6cells. $^*p < 0.05$, compared with control.

3.3 Effects of genistein on [^{3}H]nisoxetine binding to bNAT transfected COS-7 cells

As shown in Fig. 4A, the specific binding of [^{3}H]nisoxetine to bNAT in COS-7 cells was saturable. Scatchard analysis of control [^{3}H]nisoxetine binding showed a single population of binding sites with an apparent dissociation constant (K_d) of 3.10 ± 0.14 nM and maximal binding (B_{max}) capacity of 293 ± 2.8 fmol/1×10^6cells (Fig. 4B). Genistein (10 μM) increased [^{3}H]nisoxetine binding by increasing the B_{max} (350 ± 13.5 fmol/1×10^6cells) without altering the K_d value (3.53 ± 0.30 nM).

3.4 Effects of estrogen receptor antagonist and tyrosine phosphatase inhibitor on [^{3}H]NA uptake by bNAT transfected COS-7 cells

To test an involvement of estrogen receptors such as ER-α and ER-β in genistein-induced stimulation of [^{3}H]NA uptake, bNAT transfected COS-7 cells were pretreated with an inhibitor of both ER-α and ER-β, ICI 182 780, and then were incubated for another 10 min with [^{3}H]NA in the presence or absence of genistein (100 nM) or 17β-estradiol (100 nM). 17β-Estradiol (100 nM) and ICI 182 780 (100 nM) by themselves also enhanced [^{3}H]NA uptake (Fig. 5). ICI 182 780 did not inhibit but rather significantly enhanced genistein-induced [^{3}H]NA uptake, whereas stimulatory effect of 17β-estradiol plus ICI 182 780 was almost equal to that of ICI 182 780 alone. Sodium orthovanadate (50 μM), protein tyrosine phosphatase inhibitor, significantly reduced [^{3}H]NA uptake. Neither genistein nor 17β-estradiol increased [^{3}H]NA uptake in the presence of sodium orthovanadate.

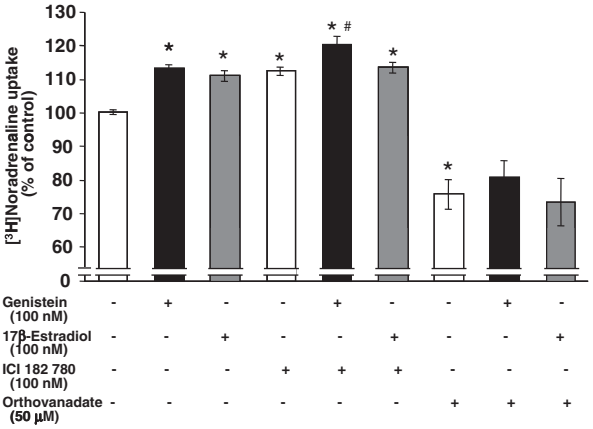

Figure 5. Effects of estrogen receptor antagonist and tyrosine phosphatase inhibitor on [^{3}H]NA uptake by bNAT transfected COS-7 cells. The bNAT transfected COS-7 cells were pretreated for 20 min with ICI 182 780 (100 nM) or orthovanadate (50 μM), and then incubated for another 10 min with [^{3}H]NA (0.1 μM, 0.1 μCi) in the presence or absence of genistein (100 nM) or 17β-estradiol (100 nM). The desipramine-sensitive [^{3}H]NA uptake by the cells was measured. Results are presented as percentage of control values. Data are means ± SEM from three separate experiments. $^*p < 0.01$, compared with control. $^\#p < 0.05$, compared with ICI 182 780 treatment.

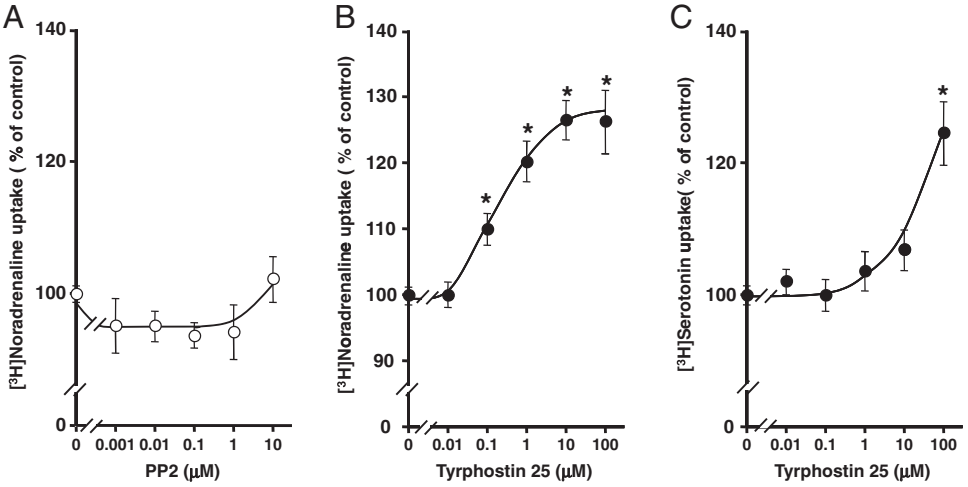

Figure 6. Effects of tyrosine kinase inhibitors on [^{3}H]NA and [^{3}H]5-HT uptake by bNAT or rSERT transfected COS-7 cells. (A) The bNAT transfected COS-7 cells were pretreated for 20 min with PP2 (0.001–10 μM), and then the desipramine-sensitive uptake of [^{3}H]NA by the cells was measured. (B) Cells were pretreated with tyrphostin 25 (0.01–100 μM) for 20 min before assessing [^{3}H]NA uptake. (C) The rSERT transfected COS-7 cells were pretreated with various concentrations of tyrphostin 25 (0.01–100 μM) for 20 min before assessing [^{3}H]5-HT uptake. Results are presented as percentage of control values. Data are means ± SEM from three separate experiments. $^*p < 0.05$, compared with control.

3.5 Effects of other tyrosine kinase inhibitors on [^{3}H]NA and [^{3}H]5-HT uptake by bNAT or rSERT transfected COS-7 cells

To test whether tyrosine phosphorylation may modulate the function of NAT in the cell, the effect of other tyrosine kinase inhibitors was studied on [^{3}H]NA and [^{3}H]5-HT uptake by bNAT or rSERT transfected COS-7 cells. PP2, which inhibits the src family of protein tyrosine kinases, failed to increase [^{3}H]NA uptake by bNAT transfected COS-7 cells (Fig. 6A). Herbimycin A, an inhibitor of p60c-src tyrosine kinase, at 100 μM also did not affect [^{3}H]NA uptake by bNAT transfected COS-7 cells (data not shown). Tyrphostin 25, an inhibitor of epidermal growth factor receptor tyrosine kinase, stimulated [^{3}H]NA uptake in a concentration-dependent manner (Fig. 6B). Moreover, tyrphostin 25 enhanced [^{3}H]5-HT uptake by rSERT transfected COS-7 cells in a concentration-dependent manner (Fig. 6C).

4 Discussion

In the present study, we demonstrated that acute treatment with genistein stimulates [^{3}H]NA uptake by noradrenergic neuroblastoma SK-N-SH cells and COS-7 cells transfected with bNAT. Genistein also enhanced [^{3}H]5-HT uptake by rSERT transfected COS-7 cells. These findings suggest that genistein increases the activity of NAT as well as SERT in the cells. A significant effect of genistein on [^{3}H]NA uptake was observed at 0.1 and 0.01 μM in SK-N-SH cells and bNAT transfected COS-7 cells, respectively. Conversely, at a high concentration of 100 μM, genistein somewhat reduced [^{3}H]NA uptake by bNAT transfected COS-7 cells. Therefore, genistein increased [^{3}H]NA uptake in a bell-shaped concentration-dependent manner. Kinetic analysis of [^{3}H]NA uptake revealed that genistein significantly increases V_{max} of [^{3}H]NA uptake without changing K_m. Furthermore, Scatchard plot analysis of [^{3}H]nisoxetine binding showed that genistein induces an increase in B_{max} of [^{3}H]nisoxetine binding with little change in K_d. These findings suggest that genistein increases the number of NATs in the cell surface membranes.

Phytoestrogens, such as isoflavones, which are structurally or functionally similar to that of 17β-estradiol, have received increasing attention for their potentially beneficial effects as estrogen agonists or anti-estrogens in health and disease. Estrogens exert multiple biological effects on a diverse array of target tissues such as the female reproductive organs. Many estrogenic actions that require from hours to days to accomplish are mediated through the nuclear estrogen receptors (ER-α and ER-β) in genomic processes [24]. In addition to the long-term action of estrogens, there is growing evidence that estrogens have nongenomic acute actions through two pathways [6, 7], *i.e.* (i) specific plasma membrane estrogen receptors such as classical nuclear estrogen receptors (ER-α and ER-β) [25, 26] and novel members of the membrane estrogen receptor family, ER-X [27] and GPR30 of the G protein-coupled receptor superfamily [28] and (ii) nonspecific and nongenomic steroid actions at high concentrations. In the present study, ICI 182 780 did not inhibit but rather enhanced NAT function induced by genistein. However, an additive effect was not observed between 17β-estradiol and ICI 182 780, an

antagonist of classical nuclear estrogen receptors (ER-α and ER-β) in regard to [^{3}H]NA uptake. Previously we reported an unique estrogen receptors in the bovine adrenomedullary plasma membranes [29] and that daidzein stimulates catecholamine synthesis *via* activation of the plasma membrane estrogen receptors in adrenal medullary cells [30]. We also showed that daidzein and genistein almost equally inhibit the binding of [^{3}H]17β-estradiol to the plasma membrane estrogen receptors [29]. In the present study, however, daidzein and coumestrol, another phytoestrogen, had little effect on NAT activity. Therefore, the present findings suggest that genistein increases NAT activity probably in a membrane estrogen receptor-independent manner, although we could not completely exclude a possibility of involvement of unknown estrogen receptors.

Genistein, a soy phytoestrogen, is a broad-spectrum inhibitor of protein tyrosine kinases, whereas daidzein is a structural analogue of genistein that lacks activity towards tyrosine kinase [31] and is often used as a negative control of genistein in this respect [13]. The inactive analogue daidzein did not affect the function of NAT. In addition, sodium orthovanadate, a protein tyrosine phospahatase inhibitor, reduced NAT activity in bNAT transfected COS-7 cells, suggesting that a modulation of protein tyrosine phosphorylation level regulates NAT activity. Furthermore, tyrphostin 25, an inhibitor of receptor-type tyrosine kinases, also increased NAT and SERT activity in bNAT or rSERT transfected COS-7 cells, whereas PP2 and herbimycin A, inhibitors of soluble-type src-family tyrosine kinases, had little effect. From these findings, it gives rise to the possibility that genistein stimulates NAT and SERT activity *via* inhibition of receptor-type tyrosine kinases in the cells. The present results are partially consistent with previous reports that tyrosine phosphorylation is involved in various regulations of Na^+/Cl^--dependent neurotransmitter transporters such as human SERT in JAR placental choriocarcinoma cells [32], γ-aminobutyric acid transporter in rat hippocampal neurons [14], and dopamine transporters in dopamine transporter-expressing *Xenopus* oocytes [33]. Furthermore, a previous study on α7 nicotinic acetylcholine receptors demonstrated that the number of functional cell surface α7 nicotinic acetylcholine receptors is rapidly controlled indirectly *via* processes involving tyrosine phosphorylation [13]. From these results, further studies are required to determine whether genistein modulates tyrosine phosphorylation in NAT proteins or other proteins associated with NAT and whether the tyrosine phosphorylation of these proteins influences NAT activity, and if so, which tyrosine kinase(s) is involved in genistein-induced up-regulation of NAT function.

Genistein is present at high concentrations as a glycoside in many soybeans and soy foods such as miso, tofu, and soy milk. The concentration of genistein in serum reached a maximum of 0.41 ± 0.06 μM at 5.9 ± 0.4 h when healthy adults ingested 100 mL of soymilk, which contained 2 μM of genistein [34]. In Japanese middle-aged women, the dietary intake of genistein was reported to be 111.6 μmol/day/capita (30.1 mg/day/capita), and the median plasma concentration of genistein was 206 nM [35]. Asian individuals generally consume more soy foods than do people in Western developed countries; the mean concentration of genistein in Japanese and UK men was 493 and 33 nM, respectively [36]. Furthermore, previous report has been shown the plasma concentration of genistein in Japanese men (276 nM) was higher than those in Finnish men (6.3 nM). Surprisingly, this report indicated that the plasma concentration of genistein was exceeded 2400 nM in one Japanese man [37]. Therefore, it is likely that the concentrations used in the present study are relevant to people's daily lives because the concentrations (0.01 or 0.1–0 μM) used in the present study partially overlap with those found in the plasma of individuals even in European countries.

The pharmacological significance of genistein-induced up-regulation of NAT and SERT activities remains to be determined. NAT and SERT are plasma membrane proteins that regulate or terminate noradrenergic and serotonergic synaptic transmission by re-uptake of NA and 5-HT from the synapse to presynaptic neurons, respectively. They also are considered as target proteins for many antidepressants that inhibit the activities of NAT and/or SERT to increase the concentrations of NA and/or 5-HT in the synapses. Therefore, it is possible that genistein influences neuronal NA and 5-HT transmission by up-regulation of NAT and SERT activities. On the other hand, soy foods and their supplements have been the subject of much interest for the reduction of menopausal symptoms such as cardiovascular diseases, including hypertension and hyperlipidemia [38, 39] and brain neuronal diseases such as depression [40, 41]. Recently, we reported that daidzein inhibits catecholamine synthesis and secretion induced by acetylcholine in cultured bovine adrenal medullary cells and proposed that daidzein may have a beneficial action on the cardiovascular system [30]. In the present study, we demonstrated that genistein stimulates NAT activity, suggesting a reduction of synaptic NA concentration in the sympathetic neurons subsequent to the suppression of cardiovascular activity. To confirm this possibility, further *in vivo* studies by administration of genistein to animals or humans are required.

In summary, we have demonstrated that genistein at a range of human serum concentrations up-regulates the activities of NAT and SERT in SK-N-SH cells and COS-7 cells transfected with bNAT and rSERT. The present findings may add new pharmacological actions for genistein on cardiovascular or neuropsychological functions and to our understanding of soy foods.

The authors are grateful to Dr. H. Bönisch (University of Bonn, Bonn, Germany) for providing the bNAT cDNA used in this study. This research was supported, in part, by Grant-in-Aids

(11839030, 20590120, 20611020) for Scientific Research (C) from the Japan Society for the Promotion of Science to Y.T., K.T., N.Y.

The authors have declared no conflict of interest.

5 References

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