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Estrogen and NMDA receptor antagonism: effects upon reference and working memory

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Abstract

Since both estrogen and NMDA receptor antagonists act on the hippocampus CA1 region and behaviorally affect hippocampal memory tasks, we examined how estrogen depletion (ovariectomy) and NMDA receptor antagonism interact upon spatial memory of the mouse. After ovariectomy or sham operation, mice were given a 2-week recovery before behavioral tests began under the influence of vehicle or (±)-3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP 2, 5 and 10 mg/kg) intraperitoneal injections. CPP is a competitive, full NMDA receptor antagonist. Spatial reference memory was tested by the water maze, spatial working memory was tested by the radial arm maze, while overall locomotive activity was monitored by the Y-maze. Results from the water maze and the Y-maze did not show any spatial reference memory or activity differences between sham-operated and ovariectomized mice. The radial arm maze, however, highlighted some working memory differences between intact and ovariectomized mice. CPP treatment impaired dose dependently –the performance of ovariectomy and sham-operated mice equally on both water maze and radial arm maze, while the drug had no effect on Y-maze performance. These results suggest that short term estrogen deprivation has no effect upon spatial –reference memory, while it impairs spatial working memory. This effect is probably not mediated by NMDA receptors.

Keywords: Estrogen; NMDA receptor antagonism; Ovariectomy; Spatial reference memory; Spatial working memory

1. Introduction

Estrogen, the female sex hormone, has effects not only on the endocrine system, but on the neurons in the brain as well. In young women, fluctuations of estrogen level due to the menstrual cycle are positively correlated with memory task performance (Hampson, 1990; Hampson and Kimura, 1988; Phillips and Sherwin, 1992). Estrogen replacement therapy in post-menopausal women improves verbal memory (Kampen and Sherwin, 1994; Sherwin, 1994, 1997; Asthana et al., 1996), and estrogen replacement has further been implicated in discouraging the onset of neurodegeneration and memory loss due to Alzheimer's Disease (Paganini-Hill and Henderson, 1994; Birge, 1997; Schneider and Finch, 1997).

Animal studies have also tied together estrogen and hippocampus-dependent memory tasks. Mimicking the post-menopausal conditions by comparing ovariectomized rats with ovariectomized plus estrogen replacement, performances on the radial arm maze and the water maze, which respectively test spatial working memory and spatial reference memory, are better in those rats receiving estradiol replacement administration (O'Neal et al., 1996; Daniel et al., 1997; Packard and Teather, 1997).

There are some cellular explanations for these estrogen-related memory improvements of rats and humans. Low levels of the estrogen derived estradiol result in low density of the synaptic spines on the hippocampus CA1 pyramidal cells in vivo (Woolley and McEwen, 1992), while in vitro estradiol administration to CA1 slices results in a significant increase of dendritic spines (Woolley et al., 1997). These fluctuations in hippocampus CA1 synaptic density occur surprisingly rapidly and naturally in experimentally unmanipulated rats during their 5-day estrous cycle (Woolley et al., 1990). Furthermore, in vitro administration of estradiol required just 48 h to increase spines (Murphy and Segal, 1996), while rats sacrificed just 1 week after ovariectomy showed significantly fewer apical

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CA1 dendritic spines than their intact counterparts (Gould et al., 1990; Woolley and McEwen, 1993).

The central players in these estradiol-linked dendrites appear to be the *N*-methyl-D-aspartate (NMDA) receptors. Of the two main glutamate excitatory receptors of the CA1 hippocampus, AMPA receptor antagonism has no effect on the estradiol-induced rise in spine density, while it is blocked by NMDA receptor antagonism (Woolley and McEwen, 1993; Murphy and Segal, 1996). These results assert that estradiol regulates synaptic spine density via NMDA receptors. Additional evidence points to an increase in the NMDA receptor itself under estradiol's influence. Binding sites for NMDA receptor agonists increases correspond with increases in estradiol level, while AMPA receptor bindings do not correlate (Weiland, 1992; Woolley et al., 1997). The NMDA receptor subunit 1 is upregulated by estradiol treatment (Gazzaley et al., 1996). This evidence led Woolley et al. (1997) to suggest that these estradiol-induced new dendritic synapses and spines could be a subpopulation in which the NMDA receptor dominates. It is therefore of particular interest to look at how NMDA receptors and estrogen influence behavior.

The best-known behavioral sphere of influence of these NMDA receptors (and also of estrogen as mentioned above) is in spatial memory. This glutamate receptor must be active in the hippocampal CA1 region in order to induce long-term potentiation and therewith the storage of information (Morris et al., 1986; Bliss and Collingridge, 1993). It is implied, therefore, that estrogen's positive effects upon memory involve the CA1 region of the hippocampus through the enhancement of its NMDA receptors.

Antagonism of the NMDA receptors produces deficits in rats for numerous hippocampus dependent tasks. Blockade of NMDA receptors causes similar behavioral deficits to lesions of the hippocampus. Both spatial reference and working memory are impaired in tests such as the water maze and the radial arm maze (Morris et al., 1986; Lyford and Jarrard, 1991; Riekkinen and Riekkinen, 1997).

Since estrogen and NMDA receptors interact on the cellular level in the CA1 region of the hippocampus, we looked for behavioral effects of this interaction on hippocampal memory tasks. We examined in the current experiment how estrogen depletion (ovariectomy) and NMDA receptor antagonism interact upon spatial memory of the mouse. Our drug of choice, (±)-3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid (CPP), is a competitive antagonist of NMDA receptors which is particularly effective in impairing short term working memory (Cole et al., 1993). Spatial reference memory was tested by the water maze, spatial working memory was tested by the radial arm maze, while overall activity was monitored by the Y-maze. Since estrogen depletion leads to fewer NMDA receptor synaptic spines in the CA1 hippocampus, we predicted that impairment of spatial memory in ovariectomized mice should require a smaller CPP dose than the threshold CPP dose for sham-operated mice.

2. Material and methods

2.1. Animals

Female C57BL/6J/Kuo mice ($n = 83$) were housed individually after operation in a controlled environment (temperature 22°C, humidity 50–60%, light schedule from 0700 h to 1900 h). Food and water were available ad libitum except for the radial-arm maze testing period. Then the mice were kept on food restriction and their weights limited to 80–90% of free-feeding weight. The study had the approval of the municipal government of Kuopio county.

2.2. Surgery

At the age of 4 months, 37 mice were ovariectomized, while 46 mice were sham-operated. Operations were performed while the mice were carefully anesthetized with Equithesin (5 ml/kg; intraperitoneally (i.p.)). All mice were given 2 weeks of recovery before the behavioral tests began. Post-mortem verification of the uterus weight confirmed the operation success.

2.3. Drug

The NMDA receptor antagonist (CPP; Research Biochemical, Natick, MA, USA) was dissolved in 0.9% NaCl and injected i.p. 25 min before the behavioral tests began. Based on preliminary studies and previous reports (Pontecorvo et al., 1991; Riekkinen et al., 1996), the drug doses used for the water maze and Y-maze were 2 and 5 mg/kg. The radial-arm maze task required CPP doses of 5 and 10 mg/kg. The control group received an equal volume of vehicle (NaCl 0.9%) as the drug groups. Throughout all behavioral tests the mice remained in the same drug group. The 2 mg/kg group for the water maze tests received 5 mg/kg during the radial arm maze tests. The 5 mg/kg group for the water maze tests received 10 mg/kg during the radial arm maze tests.

2.4. Water maze

The apparatus consisted of a black plastic pool, diameter 120 cm, with a black painted rubber square platform, 14×14 cm, located 1 cm below the water surface. This escape platform location was kept constant in the middle of the southwest quadrant. The water was kept at a constant temperature throughout the experiment (20.0 ± 1.0°C).

Initially, the mice were given 2 days of pre-training in a small pool (radius 50 cm) with the same platform (14×14 cm). This gave them experience in climbing onto the platform from the water. The mice were allowed to swim until they found the platform or for a maximum of 20 s

before they were placed on the platform for 10 s. This was repeated three times consecutively each day for the pre-training.

In the large experiment pool the starting locations, labeled North, South, East, and West, were located around the pool rim. Mice were placed in the water facing the wall at each of these starting points in a random order. The latency to find the submerged platform was timed by the experimenter, using a computer connected to an image analyzer (HVS Image, Hampton, UK) to record the swim pattern and escape latency. The mice were given 50 s to find the platform, and then were allowed to stay on the platform for 10 s. If the mouse failed to find the platform in the maximum time (50 s), the experimenter placed it there for the 10 s platform sit. The inter-trial interval was 30 s. Each mouse performed four trials/day for 5 days. During the platform training trials, escape length and percentage each animal found the platform were measured.

2.5. Y-maze

The Y-maze apparatus consisted of three compartments (10×10 cm) with connecting passages (4×5 cm) radiating out from the center. The walls were made of black plastic 10 cm high. The mouse was placed in one of the compartments and allowed to move freely for 8 min without reinforcement. An arm entry was defined as all four legs entering a compartment, and the sequence of entries was manually recorded. An alternation was defined as the entry into all three arms on consecutive choices. The number of maximum alternations was then the total number of arms entered subtracting 2, and the percent alternation was calculated as (actual alternations/maximum alternations) $\times 100$.

2.6. Radial-arm maze

The radial-arm maze used a design similar to the one developed by Olton et al. (1978) for rats and adapted to the mouse by Crusio et al. (1987). The eight arms radiated out from an octagonal Plexiglas center, 22 cm in diameter. The Plexiglas arms were 25 cm long, 6 cm wide, and 6 cm high. The arm entrances could be blocked by guillotine doors. Food pellets (Rice Krispies, Kellogg's) were placed at the inaccessible extremity of every arm behind a perforated wall to give all arms uniform scent. A single reward pellet was placed immediately in front of the perforated wall, but behind a low visual barrier (1 cm).

Mice were trained to collect food pellets from every arm of an eight-arm radial arm maze. By baiting all eight arms, an entry into an arm from which it had already retrieved the food pellet was deemed an error. Thus, performances were evaluated on the basis of two criteria: consecutive correct before the first mistake and total errors. The mice were removed from the radial arm maze after retrieval of all rewards or 16 total arm entries had been made. An entry was defined as all four paws entering the arm.

Two days of pre-training allowed the mice to explore the baited radial arm maze. Mice were placed into the center of the maze with the doors closed for 20 s. Once the doors opened, free movement followed as no doors were closed for the 7 min pre-training.

During the experimental phase, the mice were placed in the center with the doors closed for 20 s. Consequently, after each return to the center from an arm, the experimenter closed the doors for 5 s. This discouraged the mouse from utilizing mediating strategies (such as 'enter the next arm on the left'). For the radial arm maze the 14 training days were averaged for each parameter into four blocks of 3 days and the last block of only 2 days.

2.7. Statistics

The data from platform finding was normalized by using the arcussin correction before the statistical analyses. The effects of drug and operation upon the water maze performance were evaluated by analyses of variance for repeated measures on escape distance and platform finding. Y-maze performance was evaluated by an analysis of variance on total movements and percent alternation. The effects of drug and operation upon radial arm maze performance were evaluated by analyses of variance for repeated measures on errors and consecutive correct.

3. Results

3.1. Water maze

Vehicle treated mice did not differ significantly due to the sham and ovariectomy operations over escape distance

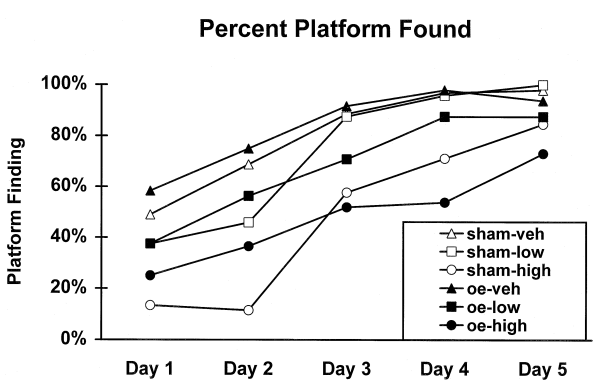

Fig. 1. Administration of CPP (2 and 5 mg/kg; i.p.) 25 min prior to daily tests impaired performance on water maze hidden-platform finding throughout the training. The CPP 5 mg/kg group improved less rapidly with training. There were no differences between sham and ovariectomized mice. The *Y*-axis represents percent of times the platform was found. The *X*-axis denotes each day's average of four training sessions. The values are daily group means.

and platform finding (ovariectomy effects: $F(1,34) < 1.5$, $P > 0.1$, for both). Both groups improved their performances over the 5-day test period (training effects: $F(1,136) > 24.1$, $P < 0.001$).

CPP administration dose dependently decreased the percentage of times the platform was found and increased the length swum compared to vehicle animals (2 mg/kg drug effect: $F(1,56) > 5.4$, $P < 0.05$; 5 mg/kg drug effect: $F(1,58) > 27.4$, $P < 0.001$). Furthermore, the CPP 5 mg/kg groups improved less rapidly in both length swum and platform finding compared to the vehicle treated mice (drug × training interaction: $F(4,232) > 2.44$, $P < 0.05$). The intact and ovariectomized mice were affected equally by the CPP 2 and 5 mg/kg injections over both parameters [drug × operation interaction ($F(1,56) < 1.2$, $P > 0.10$)]. (See Figs. 1 and 2).

3.2. Y-maze

Vehicle-treated mice did not differ significantly in total movement or in percent alternation due to the sham and ovariectomy operations (ovariectomy effect: $F(1,3) < 0.39$, $P > 0.05$) (data not shown). Neither dose of CPP (2 and 5 mg/kg) had an effect upon performance (drug effect: $F(1,2) < 0.84$, $P > 0.05$) (data not shown).

3.3. Radial-arm maze

A comparison between vehicle-treated intact and ovariectomized mice showed improvement with training for both errors and consecutive correct in both groups (training effect: $F(4,116) > 11.4$, $P < 0.001$). The ovariectomized animals did not score as well in consecutive correct as the sham-operated animals (operation effect: $F(1,30) = 5.4$, $P < 0.05$), but errors produced no difference between the intact and ovariectomized groups.

CPP 5 mg/kg had no effect on errors or consecutive correct in sham-operated or ovariectomized mice [drug effect: ($F(1,49) < 0.01$, $P > 0.10$)]. The CPP 10 mg/kg mice committed a greater number of errors (drug effect: $F(1,53) > 1.11$, $P < 0.05$) and did not improve as much as vehicle-treated mice (drug × training interaction: $F(4,212) > 3.83$, $P < 0.005$). In number of errors CPP 10 mg/kg had an equal effect on ovariectomized and intact mice (drug × operation effect: $F(1,53) = 1.1$, $P > 0.10$). Consecutive correct responses were not decreased by CPP 10 mg/kg. In consecutive correct responses the ovariectomized mice did perform significantly poorer, though the operation affected equally vehicle and CPP 10 mg/kg treated mice (operation effect: $F(1,54) = 4.1$, $P < 0.05$,

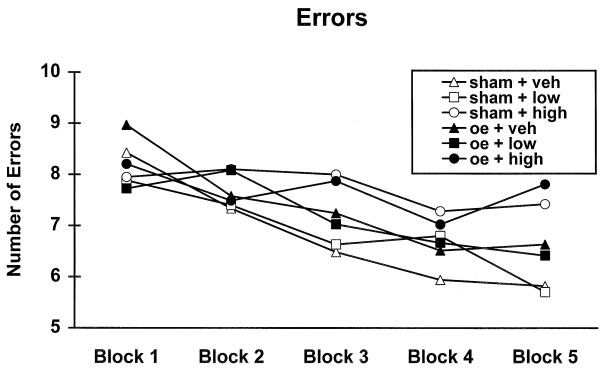

Fig. 3. Administration of CPP (5 mg/kg; i.p.) 25 min prior to daily tests had no effect upon radial arm maze errors throughout the training. The CPP 10 mg/kg group committed more errors and improved less rapidly with training. There were no differences between sham and ovariectomized mice. The *Y*-axis represents number of errors. The *X*-axis denotes averages of five blocks of 3 days each. The values are daily group means.

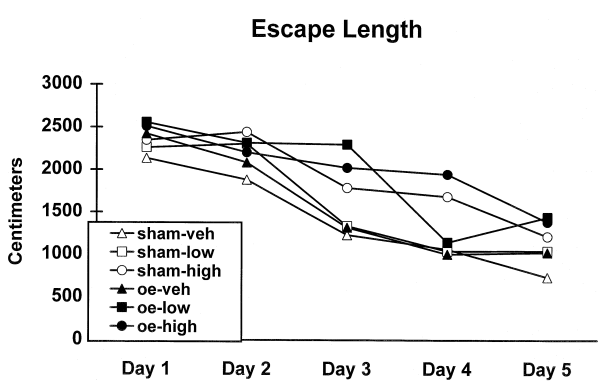

Fig. 2. Administration of CPP (2 and 5 mg/kg; i.p.) 25 min prior to daily tests impaired performance on water maze lengths swum throughout the training. The CPP 5 mg/kg group improved less rapidly with training. There were no differences between sham and ovariectomized mice. The *Y*-axis represents distance swum. The *X*-axis denotes each day's average of four training sessions. The values are daily group means.

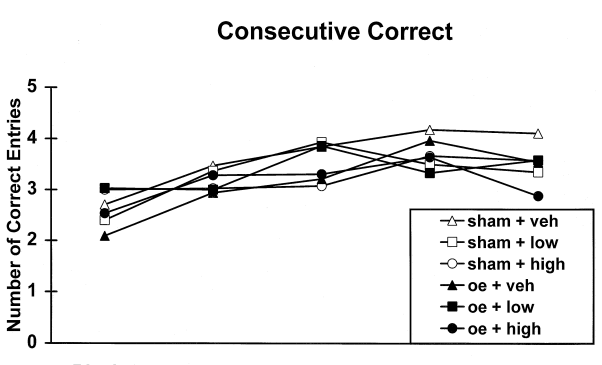

Fig. 4. Administration of CPP (5 mg/kg; i.p.) 25 min prior to daily tests did not induce earlier mistakes in the radial arm maze. The CPP 10 mg/kg group had only a trend towards earlier mistakes. Ovariectomized mice made their first mistake sooner than sham-operated mice. The *Y*-axis represents consecutive correct. The *X*-axis denotes averages of five blocks of 3 days each. The values are daily group means.

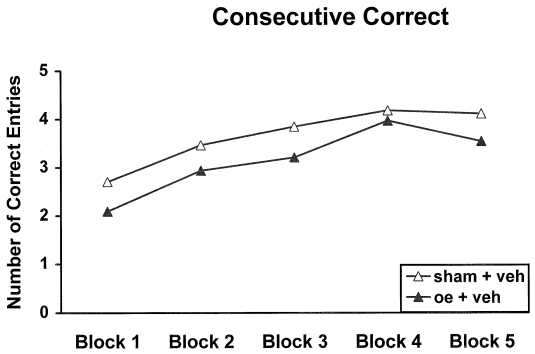

Fig. 5. For all those vehicle treated, ovariectomized mice made their first mistake sooner than sham-operated mice. The *Y*-axis represents consecutive correct. The *X*-axis denotes averages of five blocks of 3 days each. The values are daily group means.

operation × drug interaction: $F(1,54) = 1.3$, $P > 0.10$). (See Figs. 3–5.)

4. Discussion

Our results indicate that ovariectomy in mice can lead to minor deficits in spatial working memory (radial arm maze), but this operation produces no deficits in spatial reference memory (water maze) or activity (Y-maze). Our results indicate clearly that the NMDA receptor antagonist impairs spatial memory performance, but ovariectomy does not alter this CPP effect. Hence, one can conclude that ovariectomy does not provide a robust enough loss of NMDA receptor activity to allow it to be seen as a change in the dose response curve to NMDA receptor antagonists and that the spatial working memory deficit induced by ovariectomy may not result from NMDA receptor dysfunction.

Previous studies of rats have focused upon comparisons between ovariectomy and estrogen replacement. Daniel et al. (1997) administered either 30-day subcutaneous Silastic implants of 25% estradiol (in cholesterol) or 100% cholesterol to ovariectomized rats. This dose was designed to maintain 15–20 pg/ml circulating estradiol level (approximation of diestrus) throughout the testing period. Using the same consecutive correct radial arm maze parameter as our experiment, Daniel et al. (1997) also found spatial working memory differences attributed to estrogen level. They added estrogen and found improvement, while we removed estrogen and found deterioration. Our results further the assertion that spatial working memory varies directly with estrogen level.

The water maze has elicited few reference memory differences correlated to estrogen. Intact rats, compared with ovariectomized and estrogen replacement rats did not show any differences over the water maze training period (Singh et al., 1994; Simpkins et al., 1997). Our tests support the reports of equal spatial reference learning by ovariectomized and intact animals.

This accumulated evidence of course begs the question why spatial working memory but not spatial reference memory is affected by estrogen. Estrogen has widespread effects upon the brain, not limited merely to the CA1 NMDA receptor spines. Particularly notable are decreases in the cholinergic system capabilities due to ovariectomy (O'Malley et al., 1987; Singh et al., 1994; Gibbs, 1997). Acetylcholine has been linked with working memory, in particular by studies using the immunotoxin saporin. Saporin, which selectively kills acetylcholine cells, induces working memory deficits (Walsh et al., 1996), while simultaneously leaving reference memory unscathed (Shen et al., 1996). This saporin evidence is strikingly similar to the ovariectomy effects upon mouse memory and suggests that these effects may at least to some extent arise from decreased acetylcholine activity.

Our current study agrees with the previous literature that NMDA receptor antagonists (CPP specifically) impair both spatial reference and working memory, while not affecting locomotive activity (Morris et al., 1986; Lyford and Jarrard, 1991; Riekkinen and Riekkinen, 1997). Ovariectomy was not found to alter the dose response to CPP, and further the working memory effect for CPP and ovariectomy were seen on different parameters. Thus, despite the effect that both estrogen and CPP have upon NMDA receptors and working memory, they do not interact to produce behavioral differences. There are several plausible explanations for why CPP acts equally in ovariectomy and sham mice.

Firstly, ovariectomy affects other transmitter systems, such as acetylcholine (as discussed above) and serotonin (unpublished data), in addition to NMDA receptors. The ovariectomy working memory effects seen here could arise from alteration of those other systems, rather than the NMDA receptor changes. Secondly, the reference and working memory deficits induced by CPP may be mediated through actions on NMDA receptors that are not sensitive to ovariectomy.

Thirdly, it must remembered that only one NMDA receptor antagonist was used in this study. Numerous studies have reported differing behavioral effects of different NMDA receptor antagonists (Ward et al., 1990; Cole et al., 1993). Thus, it is possible that other NMDA receptor antagonists (such as a non-competitive variety) would interact with estrogen depletion. However, both competitive and non-competitive NMDA receptor antagonists have been found to impair spatial learning (Caramanos and Shapiro, 1994), and both also inhibit estradiol's effect on spine density (Woolley and McEwen, 1994). We chose CPP as the antagonist because its effects upon spatial-memory are well-characterized from previous studies and because it is a competitive antagonist and therefore more appropriate for the repeated daily exposure.

Fourthly, the NMDA receptor decrease due to ovariectomy may not be severe enough in brain areas relevant to spatial learning and memory (such as the hippocampus

CA1) to be noticed by the CPP tests. Indeed, Woolley and McEwen (1992) note only partial decreases in NMDA receptor spines of the CA1 during low estrogen periods. Perhaps a comparison between intact, ovariectomized, and ovariectomized plus estrogen replacement groups of mice would produce sufficient differences to influence the dose response to NMDA receptor antagonists.

Mice tested 2 weeks after ovariectomy do not exhibit spatial reference memory deficits, while they do show some spatial working memory deficits. These deficits most probably do not arise from NMDA receptor depletion. These results lend behavioral evidence to the histology-based assertion of Woolley and McEwen (1994) that NMDA receptor antagonism could represent an inhibition of estradiol's protective effect on spine density, rather than simply additive effects of two separate mechanisms.

In the cholinergic system, ovariectomy has both immediate and long-term deterioration effects (Gibbs, 1998; Gibbs and Aggarwal, 1998). Indeed, the memory losses associated with Alzheimer's Disease and post-menopausal women involve a long-term process. With this in mind, it is of interest to examine the effects of long-term estrogen deprivation (by ovariectomy) upon the NMDA receptor and cholinergic memory systems, which may differ from the resulting short-term behavioral effects seen here.

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