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Participation of different 5-HT receptors in the memory process in rats and its modulation by the serotonin depletor p-chlorophenylalanine

Vesselin D. Petkov, Stilyana Belcheva, Elena Konstantinova and **Roumen Kehayov**

Institute of Physiology, Bulgarian Academy of Sciences, 23 Acad. G. Bonchev St., 1113 Sofia, Bulgaria

Abstract. The memory effects of agonists and antagonists of some serotonin (5-HT) receptor subtypes were examined in experiments on rats using an active avoidance method (shuttle-box). The 5-HT receptor antagonists NAN 190 (1 mg/kg i.p.) and pindolol (6 mg/kg i.p.) improved some indices for memory; the 5-HT_2 and 5-HT_3 receptor antagonists ritanserin (1 mg/kg i.p.) and ondansetron (0.1 mg/kg i.p.) exerted a favourable effect on the mastering of active avoidance performance. The tryptophan hydroxylase inhibitor para-chlorophenylalanine (300 mg/kg i.p.) alone produced no significant changes in the indices for retention of learned behaviour but in combination with the 5-HT-receptor agonists and antagonists influenced some of their effects. The results obtained show different participation of 5-HT_{1A}, 5-HT_2 and 5-HT_3 receptors in the mechanisms of the memory process; the nature of this involvement is modulated by the brain level of serotonin.

Key words: learning, memory, shuttle-box, serotonin receptors, para-chlorophenylalanine, buspirone, NAN 190, pindolol, ritanserin, ondansetron

INTRODUCTION

Recently, a growing body of research has focused on the participation of serotonin (5-HT) in the neurochemical mechanisms of cognition and especially of learning and memory. Earlier studies, in which attempts were made to increase the brain 5-HT function by different drugs, showed that the changes in this function led, in the majority of cases, to an impairment of learning and memory (Hunter et al. 1977, Fibiger et al. 1978, Ögren et al. 1981). Surprising enough, however, was the observation that inhibitors of 5-HT uptake known to increasethe intrasynaptic concentration of 5-HT (which is believed to result in an increase of 5-HT activity - Fuller et al. 1974) improved the memory of mice (Altman et al. 1984, Flood and Cherkin 1987). The results from experiments aimed at impairing 5-HT function are also contradictory (Costall and Naylor 1992). Post mortem analyses of brains of patients with Alzheimer's type senile dementia have shown decreased levels of 5-HT and 5-hydroxy-indole acetic acid (5HIAA); biopsies of the brain tissue of such patients has revealed reduced uptake and release of 5-HT. A causal relationship between these changes and cognition impairment has not yet been established (Gower 1992). In previous experiments, we found that the age-determined memory deficit in rats (Petkov 1985, Petkov et al. 1993) was accompanied by a decreased 5-HT level and reduced number (B_{max}) and affinity (increased K_d) of 5-HT receptors (Petkov et al. 1988) in several brain structures. These results suggest that the decreased capacity of the brain serotonergic system, while not the only factor for the age-related memory impairment, plays an important role in the neurochemical changes underlying the memory deficit in aging.

Many factors may contribute to the controversial role of serotonergic neurotransmission in the cognitive process. The diversity in the functions of 5-HT receptor subtypes leads to large differences in behavioural responding due to the predominant involvement of one or another receptor subtype under the selective impact of some factors. The varying functional capacity of serotonergic neurotransmission, the disturbances in the balance between serotonergic and other transmitter systems, and the different behavioural tests make it very difficult to decide which of the components of serotonergic neurotransmission are involved in the neurochemical mechanisms of cognition.

The present study was based on two considerations. On the one hand, we set ourselves the task of obtaining additional data about the participation of the different 5-HT receptor subtypes in the mechanisms of the memory process, using various agonists and antagonists. On the other hand, we decided to further elucidate the role of the changes in the functional potential of serotonergic neurotransmission as a whole for the sensitivity of the different 5-HT receptor subtypes, and for the behavioural effects of the altered sensitivity of these receptors, by studying the changes in the behavioural effects of the 5-HT receptor agonists and antagonists used on the background of para-chlorophenylalanine (PCPA)-decreased level of brain serotonin.

METHODS

Animals and treatments

Male Wistar rats weighing 200-250 g were used. The rats had free access to standard laboratory food and water. They were housed in plastic cages (10 animals per cage) in a room maintained at constant temperature (21-22 C) and 12L:12D cycle. The experiments were performed between 10 a.m. and 1 p.m. The 5-HT receptor agonists and antagonists were administered alone or on the background of 5-HT depletion produced by the tryptophan hydroxylase inhibitor para-chlorophenylalanine (PCPA) and their behavioural effects were examined. Rats were trained to perform a reinforced two-way active avoidance task. Each drug was tested on 7-8 rats. The rats were assigned to the groups randomly. A total of 94 animals was used.

Drugs

Para-chlorophenylalanine (PCPA), 300 mg/kg, was injected intraperitoneally as a Tween-80 sus-

pension 24 h before the 5-HT receptor agonists and antagonists, i.e., 24 h and 30 min before the first shuttle-box-training; buspirone (Sigma Chemical Co., St. Louis, Missouri, USA), NAN 190 - 1-(2-methoxyphenyl)-4-/4-(2-phthalimido) butyl/-piperazine hydrobromide (Dept. of Medicinal Chemistry, Duphar, Amsterdam), pindolol (Sandoz Ltd., Basel, Switzerland), ritanserin (Janssen, Beerse, Belgium), and ondansetron (Glaxo, Hertfordshire, UK) were injected intraperitoneally as Tween-80 suspensions 30 min before shuttle-box training and 30 min before retention testing.

Two-way active avoidance - shuttle-box

The apparatus was a box (50 x 29 x 21 cm) divided in two equal compartments provided with a round opening at the centre. Light (21 W switched on alternately in the two compartments) was used as conditioned stimulus (CS). The CS was lighted in the opposite part of the cage in which the rat was located at the end of the inter-trial period. The unconditioned stimulus (US) was an electric scrambled shock (0.5 mA, 50 Hz) applied to the grid floor for 12 s. The CS preceded the onset of the US by 9 s and continued during the action of the US. An avoidance response was recorded when the animal avoided the US within 9 s after the onset of the CS. An avoidance response terminates CS and an escape response terminates CS + US. The inter-trial interval was 9 s. When the rat did not enter the opposite compartment of the shuttle-box during the whole period of action of the CS and US the response was considered inadequate (Ia), i.e., a "fail to escape" response. Before starting the experiments each rat was adapted to the shuttle-box; to this end it was placed in the apparatus for 6 min and 20 stimuli were applied (alternating 9 s light and 9 s interval) without electrical reinforcement. On the next day each rat was trained with 50 trials. Twenty four hours later the rat was trained again with 50 trials. A retention test was given 24 h after the second training session: the light stimulus was applied for 9 s and was followed by 2 s electric shock. The inter-trial interval was 9 s.

In some experiments, we observed sporadic inter-trial cage crossings which were not punished.

Eight shuttle-box apparatuses were placed in a sound-proof room and each apparatus was sound--proofed. The data from each shuttle-box were fed into an analog-to-digital converter coupled to a computer with appropriate on-line program. Each avoidance and each inadequate response ("fail to escape" response) was recorded on a printer.

Statistics

The data were processed by analysis of variance (ANOVA), followed by Student's *t*-test for post hoc group comparisons.

RESULTS

Separate analyses of variance (ANOVA) were used to analyse the data (avoidances and inadequate responses) obtained during each of the two training days and the time of retention testing. ANOVA analyses of the avoidance responses revealed on the 1st training day significant effects due to ondansetron and pindolol (respectively $F(1;31)=13.23$, $P<0.001$ and $F(1;31)=12.36$, $P<0.01$) and a significant PCPA x ondansetron interaction ($F(1;31)=4.19$, $P<0.05$). On the 2nd training day there were significant effects due to ondansetron and ritanserin (respectively $F(1;31)=14.99$, $P<0.001$ and $F(1;30)=8.48$, $P<0.01$) and a significant PCPA x buspirone interaction ($F(1;31)=4.18$, $P<0.05$). At the time of the memory test there were significant effects due to ondansetron, ritanserin, pindolol and NAN 190 (respectively $F(1;31)=25.78$, $P<0.001$; $F(1;30)=4.33$, $P<0.05$; $F(1;31)=10.41$, $P<0.01$ and $F(1;30)=7.39$, $P<0.01$) and a significant PCPA x NAN 190 interaction ($F(1;30)=11.36$, $P<0.01$).

ANOVA analyses of the inadequate responses revealed on the 1st training day significant effects due to buspirone and NAN 190 (respectively $F(1;31)=24.61$, $P<0.001$ and $F(1;30)=11.40$, $P<0.01$) and a significant PCPA x NAN 190 interaction ($F(1;30)=9.80$, $P<0.01$). On the 2nd training day the only significant effect was due to pindolol

TABLE I

Effects of PCPA, Buspirone (Busp), NAN 190 (NAN), Pindolol (PIND), Ritanserin (RIT), Ondansetron (OND) administered alone or combined with PCPA on the number of avoidances in a shuttle-box

Experimental groups	Avoidances ± SEM		
	1st day	2nd day	Retention
Saline (n=8)	2.0±0.7	1.6±0.8	3.6±1.6
PCPA + Saline (n=8)	1.0±0.5	3.1±0.6	4.8±1.6
Buspirone (n=8)	3.0±0.8	3.4±1.5	5.1±1.8
PCPA + Busp. (n=8)	1.6±0.4	1.2±0.2*	4.0±0.8
NAN 190 (n=8)	2.6±0.5	3.5±1.1	14.6 ± 2.3^{oo}
PCPA + NAN (n=7)	2.3±0.6	3.1±1.3	3.6±1.5**
Pindolol (n=8)	5.4 ± 1.2^{oo}	1.9±0.8	13.6 ± 3.7^{oo}
PCPA + PIND (n=8)	3.4±0.7	6.9±1.9	11.0±2.3
Ritanserin (n=8)	3.5±1.1	5.0 ± 1.3^{oo}	11.0 ± 4.5^{o}
PCPA + RIT (n=7)	3.0±0.9	8.2±1.9	9.1±2.4
Ondansetron (n=8)	5.1 ± 0.6^{ooo}	5.8 ± 1.3^{ooo}	11.8 ± 2.2^{ooo}
PCPA + OND (n=8)	1.9±0.5*	8.6±1.9	22.0 ± 3.9^{oo}

$^{o}P<0.05$, $^{oo}P<0.01$, $^{ooo}P<0.001$ - significance of difference vs. controls, $*P<0.05$, $**P<0.01$ - significance of difference vs. the effect of the agonist or antagonist administered alone, n = number of rats per group.

($F(1;31)=10.45$, $P<0.01$). At the time of the memory test there were significant effects due to buspirone and ondansetron ($F(1;31)=4.83$, $P<0.05$ and $F(1;31)=4.37$, $P<0.05$). On the second training day and at the memory test no significant interactions were noted.

Post hoc analysis of the data revealed that parachlorophenylalanine (PCPA) at a single dose of 300 mg/kg injected intraperitoneally (i.p.) 24 h before shuttle-box training tended to increase the number of avoidances on the second training day and upon retention testing 24 h after training as compared to controls (Table I).

Buspirone (1 mg/kg i.p.) also tended to increase the number of avoidances on the second training day and upon retention testing as compared to controls (Table I) and, in the majority of cases, this was accompanied by a great increase in the number of inadequate responses (significant on the first training day - Table II).

Particularly pronounced were the effects of the 5-HT receptor antagonist NAN 190 (1 mg/kg i.p.). The impaired performance on the avoidance task at the first stage of training (a significant increase in the number of inadequate responses on the first training day as compared to controls) was followed by a marked improvement of avoidance behaviour upon retention testing 24 h after training (a significant increase in the number of avoidances and a decrease of inadequate responses as compared to controls - Tables I and II).

Pindolol (6 mg/kg i.p.) had a clear memory- and learning-improving effect. On the first training day and upon retention testing 24 h after training pindolol-treated rats showed an increased number of avoidances and a decreased number of inadequate responses as compared to controls (Tables I and II).

Learning and memory processes were also facilitated by the 5-HT_2 and 5-HT_3 receptor antagonists. Both ritanserin and ondansetron increased the number of avoidances on the first and second training days and upon retention testing as compared to controls (Table I). In ondansetron-injected rats the number of inadequate responses was decreased as

TABLE II

Effects of PCPA, Buspirone (Busp), NAN 190 (NAN), Pindolol (PIND), Ritanserin (RIT), Ondansetron (OND) administered alone or combined with PCPA on the number of inadequate responses in a shuttle-box

Experimental groups	Avoidances ± SEM		
	1st day	2nd day	Retention
Saline (n=8)	3.1±1.1	2.0±0.9	4.1±1.5
PCPA + Saline (n=8)	1.5±0.6	3.0±0.8	3.4±1.3
Buspirone (n=8)	18.6±3.6^{ooo}	4.5±2.6	8.0±3.0^{o}
PCPA + Busp. (n=8)	9.3±2.8	7.5±2.8	8.3±1.7
NAN 190 (n=8)	16.4±3.7^{oo}	3.3±1.5	1.4±0.7
PCPA + NAN (n=7)	2.0±0.7**	4.3±2.3	3.1±1.4
Pindolol (n=8)	2.0±0.9	0.6±0.4^{oo}	2.4±1.3
PCPA + PIND (n=8)	0.1±0.1	0.1±0.1	1.5±0.5
Ritanserin (n=8)	3.8±1.3	3.4±1.9	3.3±1.8
PCPA + RIT (n=7)	2.3±1.6	0.1±0.1	4.4±2.5
Ondansetron (n=8)	1.6±0.5	1.8±0.8	0.6±0.4^{o}
PCPA + OND (n=8)	0.6±0.4	0.3±0.2	2.0±1.3

$^{o}P<0.05$, $^{oo}P<0.01$, $^{ooo}P<0.001$ - significance of difference vs. controls, $^{**}P<0.01$ - significance of difference of the effect of PCPA + NAN vs. NAN, n=number of rats per group.

compared to that in controls (significantly upon retention testing Table II). Post hoc analysis of the data indicated that the PCPA-induced depletion of brain 5-HT resulted in changes in some of the behavioural effects of the 5-HT-receptor agonists and antagonists (Tables I and II).

On the background of PCPA buspirone tended to decrease the number of avoidances (Table I). On the first training day the number of inadequate responses of the PCPA + buspirone-treated rats was also decreased as compared to that in the rats injected with buspirone only (Table II).

Following PCPA-induced depletion of brain 5-HT NAN 190 failed to show its effect, i.e., a great increase in the number of avoidances upon retention testing 24 h after training and in the number of inadequate responses on the first training day (Tables I and II).

In the pindolol + PCPA-injected rats there was a tendency towards decreasing the number of avoidances on the first training day and upon retention testing 24 h after training as compared to the rats that were given only pindolol. On the second training day, however, pindolol significantly increased avoidances and this effect was more pronounced than the effect it exerted when applied alone (Table I).

The presence of PCPA + ritanserin tended to decrease the number of avoidances on the first training day and upon retention testing and to increase it on the second training day (Table I) when the number of inadequate responses was reduced to zero (Table II).

The combination PCPA + ondansetron increased the number of avoidances on the second training day and upon retention testing (significantly upon retention testing) but on the first training day it was significantly smaller than that in the rats treated with ondansetron only (Table I). The number of inadequate responses in the PCPA + ondansetron-treated rats was decreased on the first and second training days (significantly on the first training day) and increased upon retention testing as compared to that in the rats that were given only ondansetron (Table II).

DISCUSSION

During recent years a number of 5-HT receptor subtypes having different roles in the functions of serotonergic neurotransmission, including the functions connected with learning and memory processes, have been described. As yet, however, the data available are fragmentary and rather contradictory.

The present study was aimed at examining the effects of agonists and antagonists of several 5-HT receptor subtypes on the performance of a punishment-reinforced active avoidance task, when applied alone or on the background of decreased (by the tryptophan hydroxylase inhibitor PCPA) functional capacity of serotonergic neurotransmission. Our results, in agreement with others (Vanderwolf 1987) demonstrated that the PCPA-induced depletion of brain 5-HT produced no significant changes in the active avoidance responding reflecting changes in the memory process.The data concerning changes in the behavioural indices of memory induced by selective agonists and antagonists of different 5-HT receptor subtypes raise the possibility that changes in serotonergic neurotransmission on the whole may not be responsible for changes in memory, but rather disturbances in the functional balance between the components of this brain neurotransmitter system.

The 5-HT_{1A} receptor agonist buspirone induced behavioural changes (an increase in the number of inadequate responses, Table II) which might be interpreted as reflecting impairments of learning and memory.

NAN 190 produced great increase in the number of avoidances upon retention testing 24 h after shuttle-box training as compared to controls, which was indicative of the memory-facilitating effect of this 5-HT receptor antagonist. However, the significantly increased number of inadequate responses on the first training day as compared to controls suggested a delay in retention of the active avoidance task. Pindolol, another agent with antagonistic action on 5-HT_{1A} receptors, exerted an effect analogous to that of NAN 190, namely, it significantly increased the number of avoidances upon retention testing 24 h after training. But pindolol increased the number of avoidances on the first training day as well. These effects of the agent known to possess not only a beta-adrenoceptor-blocking action but also a high affinity to brain 5-HT_{1A} receptors (Hoyer 1988) could reflect an improvement of learning and memory processes.

The increased number of avoidances on the second training day and upon retention testing 24 h after training in the rats treated with the 5-HT_2 receptor antagonist ritanserin suggested its favourable effect on the performance of the active avoidance task.

Other authors (Altman and Normile 1986, 1987, Normile and Altman 1987, Strek et al. 1989) reported that the selective 5-HT2 receptor antagonists pirenperon, ketanserin, and mianserin could enhance the memory of a previously learned inhibitory avoidance in mice. It was, however, found that the effects of the antagonists depended on the time of their administration: i.e., an improvement of memory following post-training administration and a dose-dependent impairment of retention following pre-training administration (Altman and Normile 1986).

In the present experiments the 5-HT_2 receptor antagonist ritanserin was administered before the first training day and after the second training day prior to retention testing (24 h after the second training day). Thus retention was tested following pre- and post-training administration of the 5-HT_2 receptor antagonist and the data about improvements of memory (a larger number of avoidances compared to controls) suggested an analogy with the memory-facilitating effect of the 5-HT_2 receptor antagonists when administered after the training session. Worthy of note was the finding that improved retention of learned behaviour (a significantly larger number of avoidances as compared to controls - Table I) was observed also on the second training day, that could be considered retention testing 24 h after the first training day prior to which ritanserin was administered. In other words, in the two-way active avoidance situation the 5-HT recep-

tor antagonist ritanserin improved or tended to improve retention following pre- and post-training administration.

The 5-HT_3 receptor antagonist ondansetron showed pronounced memory-facilitating effects: on the first and second training days and upon retention testing the number of avoidances was significantly increased and the number of inadequate responses was decreased as compared to controls (Tables I and II).

In related work (unpublished data), we found that the 5-HT_3 receptor antagonist MDL 72222 improved memory (an increased number of avoidances and a decreased number of inadequate responses compared to controls) while the 5-HT_3 receptor agonist 2-methyl-5-HT impaired the retention of learned behaviour (a decreased number of avoidances and an increased number of inadequate responses compared to controls) in shuttle-box-trained rats.

Other authors also reported a favourable effect of 5-HT_3 receptor antagonists on learning and memory (Barnes et al. 1990, Crook and Lakin 1991, Greenshow 1993), which was associated with the facilitating influence they exert on cholinergic function through elimination of serotonin's inhibitory control over acetylcholine release (Vizi et al. 1981, Robinson 1983, Gillet et al. 1985, Barnes et al. 1986, Bianchi et al. 1990, Maura et al. 1992).

Since the works of Tenen (1967) and Brody (1970) demonstrating that PCPA facilitates learning, many authors have found that the favourable effect of this depletor of brain 5-HT on learning and memory processes depends on the behavioural task and the experimental procedure (Koler and Lorens 1978, Ögren et al. 1981).

The PCPA-induced depletion of brain 5-HT (Jequier et al. 1967) in the two-way punishment-reinforced active avoidance situation led to significant changes in the rat's behavioural responses to the 5-HT receptor agonists and antagonists, which might be considered indices for the memory process (Tables I and II). Obviously, on the background of induced 5-HT depletion in the synaptic region there occur changes in the sensitivity of 5-HT receptors (most probably as a result of up-regulation), which determine the altered behavioural effects of the agonists and antagonists of the different 5-HT receptor subtypes.

The rats treated with buspirone + PCPA tended to decrease the number of avoidances on the first and second training days and upon retention testing as compared to the rats that were given only buspirone. This suggests a tendency towards decreasing of the memory effect of buspirone under conditions of reduced functional capacity of serotonergic neurotransmission. At the same time, the combination buspirone + PCPA significantly decreased the number of inadequate responses on the first training day as compared to that when buspirone was applied alone. This speaks for the favourable role of the 5-HT_{1A} receptor agonist in the acquisition of an active avoidance task in the case of 5-HT depletion.

The combination of the 5-HT1A receptor antagonist NAN 190 with PCPA significantly decreased the number of avoidances upon retention testing 24 h after training as compared to that in rats treated with NAN 190 only (Table I), making it equal to that in the controls. This finding suggests a role of the decreased brain level of 5-HT in the abolishment of the favourable effect of NAN 190 on the retention of learned behaviour.

The other 5-HT_{1A} receptor antagonist pindolol, which lacks selectivity and is also active on other receptors, especially on the beta-adrenoceptor and the 5-HT_{1B} receptor, preserved its innate favourable effect on memory (an increase in the number of avoidances upon retention testing as compared to controls). However, under conditions of PCPA-induced depletion of brain 5-HT, pindolol also significantly increased the number of avoidances on the second training day as compared to controls and to rats treated with pindolol only (Table I). Obviously, the decline in the 5-HT level in the synaptic area potentiates the favourable effect of pindolol. The difference in the effects of pindolol and NAN 190 might be due to the multireceptor action of pindolol. When interpreting the data about the effect of pindolol on the memory process, one should consider the role of the interactions between serotonergic

and noradrenergic neurotransmitter systems for cognition. Some studies suggest functional reciprocal relationships between the two systems. Anatomical relationships are indicated by the identification of 5-HT nerve terminals in locus coeruleus (for review see Mc Entee and Crook 1991). The alpha-adrenoceptor agonist clonidine at doses of 0.1 to 1 mg/kg increases the firing of 5-HT cells in the dorsal raphe nucleus by activating a facilitatory adrenergic influence on the 5-HT neurones (Svensson et al. 1975). Such a facilitatory adrenergic influence has also been reported by Gallager and Aghajanian (1976) and by Baraban and Aghajanian (1980). We have studies to show that the 5-HT2 receptor antagonist ketanserin prevents the clonidine-induced performance deficit in the passive avoidance situation in rats (Genkova-Papasova et al. 1994). Based on these data, one could suggest that in the case of decreased serotonin (on the background of PCPA--induced 5-HT depletion), the changes occurring in the responsiveness of 5-HT receptors determine new interactions between the brain serotonergic and adrenergic mechanisms. This, in turn, results in changes in the effects of pindolol which interacts with serotonergic and noradrenergic receptors. Instead of tending to impair acquisition, pindolol improved it (a larger number of avoidances on the second training day when pindolol was administered in the presence of PCPA as compared to the small number of avoidances when given alone - Table I).

Interesting results were obtained with the 5-HT_2 receptor antagonist ritanserin. No significant difference in the number of avoidances was observed between the PCPA + ritanserin-treated rats and the rats that were given only ritanserin, but the number of inadequate responses in the PCPA + ritanserin--treated rats was reduced to zero on the second training day. This suggests that the absence of a clear cut influence of the decreased brain level of 5-HT on the effect of ritanserin on the acquisition of the active avoidance task is accompanied by an amelioration of the mastering of this task.

In the shuttle-box experiments the 5-HT_3 receptor antagonist ondansetron, attenuating the 5-HT receptor influence, increased the number of avoidances and decreased the inadequate responses on the first and second training days and upon retention testing as compared to controls. After 5-HT deprivation the memory-facilitating effect of ondansetron was enhanced; the PCPA + ondansetron--treated rats showed a larger number of avoidances upon retention testing as compared to rats treated with ondansetron only and control rats (Table I), suggesting the dynamically changing modulating influence of the 5-HT_3 receptor on memory. The memory-impairing effect of the 5-HT_3 receptor was particularly pronounced in the case of 5-HT decrement in rats trained to perform an active avoidance task (upon retention testing the number of avoidances in the PCPA + ondansetron-treated rats was larger than that in the controls and in the rats treated either with PCPA or with ondansetron only - Table I).

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