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Inhibition by L-3,4-dihydroxyphenylalanine of hippocampal CA1 neurons with facilitation of noradrenaline and γ-aminobutyric acid release

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Abstract

Electrophysiological studies were performed to elucidate whether L-3,4-dihydroxyphenylalanine (L-DOPA) acted on hippocampal CA1 neurons, since this drug has been reported to act as a neurotransmitter in the hypothalamus and striatum. Hippocampal slices (450 μm thick) obtained from male Wistar rats (4–7 weeks of age) were placed in a bath (maintained at 30 ± 1°C) continuously perfused with artificial cerebrospinal fluid. The population spikes elicited by electrical stimuli applied to the Schaffer collateral/commissural fibers were recorded in the hippocampal CA1 region, using a glass micropipette filled with 3 M NaCl. Drugs were applied in the bath through a perfusion system. The population spikes were inhibited by L-DOPA (1 nM–10 μM) with a bell-shaped concentration–response curve ($n = 7–15$). Maximum inhibitory effects were obtained at 100 nM. L-DOPA cyclohexyl ester, a putative L-DOPA recognition site antagonist, antagonized the L-DOPA-induced inhibition of population spike. However, the inhibition remained unaffected in the presence of 3-hydroxybenzylhydrazine, an aromatic amino acid decarboxylase inhibitor. Furthermore, bath application of either phentolamine, an α-adrenoceptor antagonist, or bicuculline, a $GABA_A$ receptor antagonist, antagonized the inhibitory effects of L-DOPA on population spikes. In addition, bicuculline (1 μM) antagonized the inhibition of population spike induced by 6-fluoronorepinephrine (10 μM), an α-adrenoceptor agonist, while phentolamine (10 μM) did not affect the muscimol (1 μM)-induced inhibition. These results suggested that L-DOPA itself acted on L-DOPA recognition sites to release noradrenaline, and that the latter facilitates γ-aminobutyric acid (GABA) release via α-adrenoceptors located on the GABA-containing cells and/or their nerve terminals, thereby inhibiting the population spikes in the hippocampal CA1 field. © 2001 Published by Elsevier Science B.V.

Keywords: L-DOPA (L-3,4-dihydroxyphenylalanine); L-DOPA cyclohexyl ester; 3-Hydroxybenzylhydrazine; Noradrenaline; GABA (γ-aminobutyric acid); Population spike; CA1 region

1. Introduction

L-3,4-Dihydroxyphenylalanine (L-DOPA) has been widely used for the treatment of Parkinson's disease. However, this drug has been reported to be a neurotransmitter and/or neuromodulator in the brain (Misu and Goshima, 1993) based on the following observations. First, both L-DOPA as an end product and its synthetic enzyme have been detected in some areas of the brain such as dorsal vagal complex, ventral tegmental area, substantia nigra, hypothalamus, and amygdala (Jaeger et al., 1984; Kitahama et al., 1988, 1990; Mons et al., 1988; Tison et al., 1989). Second, endogenous L-DOPA is released by depolarizing stimuli (electrical field stimuli or high K^+ solution) in rat striatal slices and in vivo in a Ca^{2+}- and concentration-dependent manner (Goshima et al., 1985, 1993; Nakamura et al., 1992). Third, application of L-DOPA to organ baths in a nanomolar concentration stereo-selectively facilitates the release of noradrenaline and dopamine from rat hypothalamic and striatal slices in the presence and absence of aromatic amino acid decarboxylase inhibitor (Goshima et al., 1986, 1990a). Fourth,

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under conditions of inhibition of aromatic amino acid decarboxylase, microinjection of L-DOPA in the rostral ventrolateral medulla produces hypertension and tachycardia (Kubo et al., 1992; Yue et al., 1993). These responses, including the L-DOPA-induced release of catecholamines, are blocked by L-DOPA methylester or L-DOPA cyclohexyl ester, both of which are competitive antagonists for the binding site of L-DOPA (Furukawa et al., 2000; Goshima et al., 1990b, 1999a; Misu et al., 1997). In addition, a high concentration (micromolar concentration) of L-DOPA stereo-selectively releases glutamate from striatal slices in the presence of aromatic amino acid decarboxylase inhibitor, and this effect is blocked by L-DOPA methylester (Goshima et al., 1993).

It is well-known that the hippocampus receives a dense innervation of noradrenergic fibers from the locus coeruleus, and is inhibited and excited by α- and β-adrenoceptor activation, respectively (Curet and De Montigny, 1988; Frankhuyzen and Mulder, 1982; Mueller et al., 1982). The noradrenergic system is important in the induction of long-term potentiation in the hippocampus (Sakai et al., 1991). In addition, L-DOPA has been reported to release noradrenaline in hypothalamic slices (Sato et al., 1993). Furthermore, the existence of a Na^+-dependent uptake site for L-DOPA in the hippocampus was recently reported (Goshima et al., 1999; Sugaya et al., 2000). Therefore, the effects of L-DOPA on hippocampal CA1 were electrophysiologically examined using brain slice preparations to clarify whether or not L-DOPA affects noradrenergic transmission and acts as a neurotransmitter or neuromodulator in this area.

2. Materials and method

2.1. Animals

Male Wistar rats 4 to 7 weeks old (Charles River Japan, Tokyo, Japan) were used. All procedures were performed in accordance with the guidelines for use of Laboratory Animals of Hiroshima University School of Medicine.

2.2. Recording of population spikes and drug application

Extracellular recordings were made from the stratum pyramidale in the CA1 region of Wistar rat hippocampal slices. Hippocampal slices (450 μm thick) were prepared from male Wistar rats and incubated in artificial cerebrospinal fluid (ACSF) at 34°C for 1 h. ACSF contained (in mM): NaCl 113, KCl 3, NaH_2PO_4 1, D-glucose 11, $CaCl_2$ 2, $MgCl_2$ 1, $NaHCO_3$ 25. A slice was placed in a bath maintained at 30°C, into which ACSF was perfused continuously at a rate of 1.5–3 ml/min. The ACSF was continuously aerated with a gas mixture of 95% O_2 and 5% CO_2. Population spikes elicited in the hippocampal CA1 region by electrical stimuli (3–8 V, 0.1 ms duration, 0.2 Hz) applied to the Schaffer collateral/commissural fibers were recorded using a glass microelectrode filled with 3 M NaCl. Population spikes, recorded every 5 min, were averaged over the last 6 of 11 consecutive responses. The height of the population spike was measured as indicated in Fig. 1 (inset). Briefly, the amplitude of the population spike was taken as the average of the differences between the spike peak negativity and the preceding

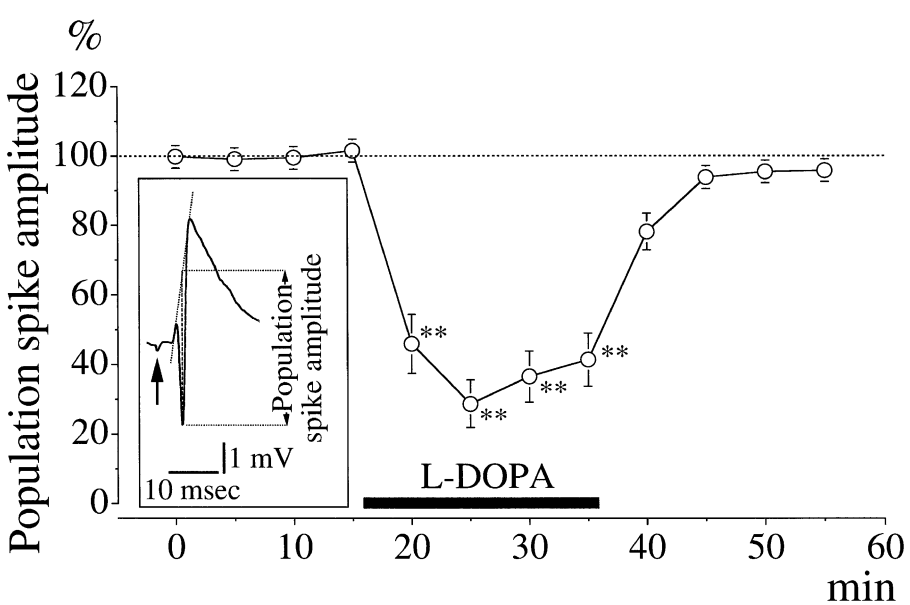

Fig. 1. The graph shows the time course of the effects of L-DOPA (100 nM) on population spikes amplitude. Symbols and bars represent the means and S.E.M., respectively ($n = 15$). Drug was applied during the period indicated by the horizontal bar in the graph. $^{**}P < 0.01$, significantly different from the value before drug application. Inset: Method of measurement of amplitude of the population spike induced by stimulation of Schaffer collateral/commissural fibers in the CA1 area. Stimulation was applied at the time indicated by the arrow.

and following positivities and determined as the length of the vertical line shown. The effects of the drugs are expressed as % relative to the mean of three population spikes just before application of the drug. Drugs were applied to the bath through the perfusion system for 15–20 min. In some experiments, the aromatic amino acid decarboxylase inhibitor, 3-hydroxybenzylhydrazine (NSD-1015), was applied to the preincubation medium and was present throughout the experiments.

2.3. Drugs

The drugs used were as follows: L-DOPA, (Nacalai Tesque, Kyoto, Japan), bicuculline methiodide and phentolamine hydrochloride (Sigma, St. Louis, MO, USA), muscimol hydrobromide and 6-fluoronorepinephrine hydrochloride (RBI, Natick, MA, USA), 3-hydroxybenzylhydrazine dihydrochloride (Aldrich, Milwaukee, WI, USA), and L-DOPA cyclohexyl ester, synthesized by Drug Discovery Laboratories, Pharmaceutical Research Institute, Kyowa Hakkou Kogyo Shizuoka, Japan. The drugs were dissolved with ACSF to the desired concentrations.

2.4. Statistical analyses

Results are expressed as means ± S.E.M. The statistical significance was assayed by a one-way analysis of variance (one-way ANOVA) test followed by Tukey honestly significant difference (Tukey HSD) test as a post-hoc analysis.

3. Results

3.1. Effects of L-DOPA

L-DOPA at concentrations of 1 nM–10 μM induced inhibition of population spikes, although no effect was observed at 100 pM. The time course of the effects of L-DOPA on the population spikes at 100 nM is shown in Fig. 1. A significant ($P < 0.01$) inhibition was observed 5 min after application of L-DOPA and recovery was seen 5 min after wash-out. The inhibitory effects of L-DOPA were dose-dependent (Fig. 2). The maximum effects of L-DOPA were observed at 100 nM; population spike amplitude was significantly ($P < 0.01$) decreased to 28.7 ± 6.8% ($n = 15$) of the control 10 min after application of 100 nM L-DOPA. L-DOPA (1 μM)-induced inhibition was also observed in the presence of NSD-1015 (20 μM), an aromatic amino acid decarboxylase inhibitor (Fig. 3). There were no significant differences in 1 μM L-DOPA-induced inhibition in the presence and absence of NSD-1015 (20 μM).

3.2. Effects of L-DOPA cyclohexyl ester on L-DOPA-induced inhibition

L-DOPA cyclohexyl ester (30 nM) alone was applied to the bath 10 min prior to application of L-DOPA (100 nM) with L-DOPA cyclohexyl ester (30 nM). Under these conditions, the L-DOPA-induced inhibition was completely blocked, although L-DOPA cyclohexyl ester alone did not affect the population spike (Fig. 4).

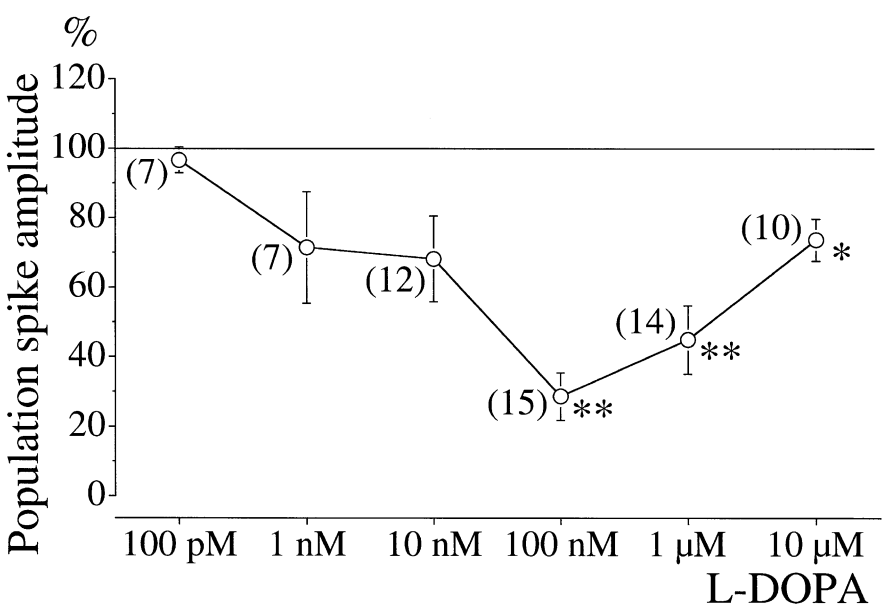

Fig. 2. Concentration–response relationship of the inhibitory effects of L-DOPA on population spikes in CA1. Symbols and bars represent the means and S.E.M., respectively ($n = 7–15$), 10 min after L-DOPA application. $^{*}P < 0.05$ and $^{**}P < 0.01$, significantly different from the value before L-DOPA application. The number in parentheses indicates the number of slices tested.

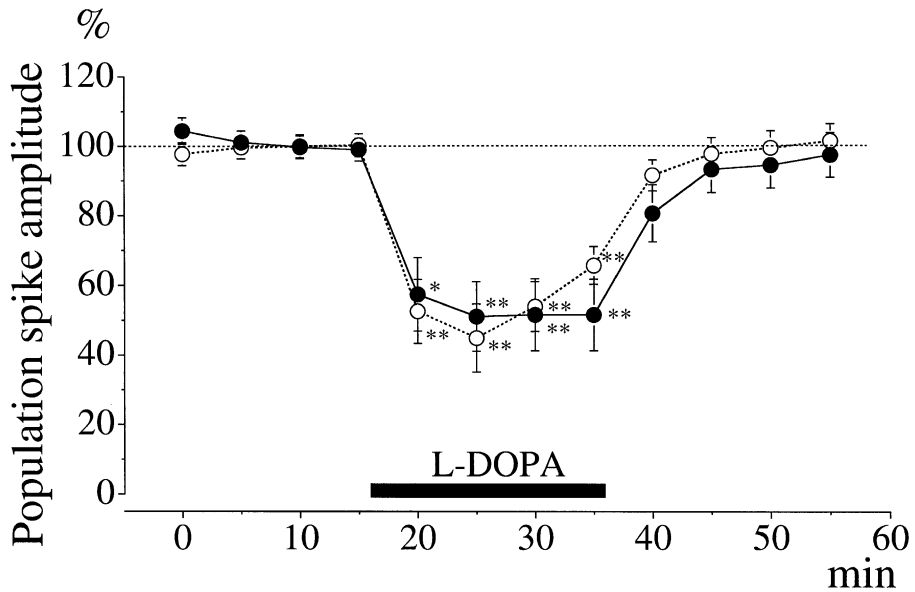

Fig. 3. Inhibitory effects of L-DOPA (1 μM) on population spikes in the presence ($n = 7$, ●) and absence ($n = 14$, ○) of NSD-1015 (20 μM), an aromatic amino acid decarboxylase inhibitor. Symbols and bars represent the means and S.E.M., respectively. L-DOPA was applied during the period indicated by the horizontal bar in the graph. $^{*}P < 0.05$, and $^{**}P < 0.01$, significantly different from the value before drug application.

3.3. Effects of α-adrenoceptor agonist and antagonist, and $GABA_A$ receptor agonist and antagonist

Application of phentolamine, an α-adrenoceptor antagonist, antagonized the L-DOPA-induced inhibition of population spike. The population spike amplitude was decreased to $19.4 \pm 15.8\%$ ($n = 3$) 15 min after application of L-DOPA (1 μM) and was significantly ($P < 0.01$) increased to $113.7 \pm 9.8\%$ ($n = 3$) 15 min after simultaneous application of L-DOPA with phentolamine (10 μM) (Table 1). Similarly, bicuculline, a $GABA_A$ receptor antagonist, blocked the L-DOPA-induced inhibition of population spike. The population spike amplitude was decreased to $46.7 \pm 14.3\%$ ($n = 6$) 15 min after application of L-DOPA (1 μM) and the amplitude was significantly ($P < 0.05$) increased to $112.5 \pm 17.5\%$ ($n = 6$) 15 min

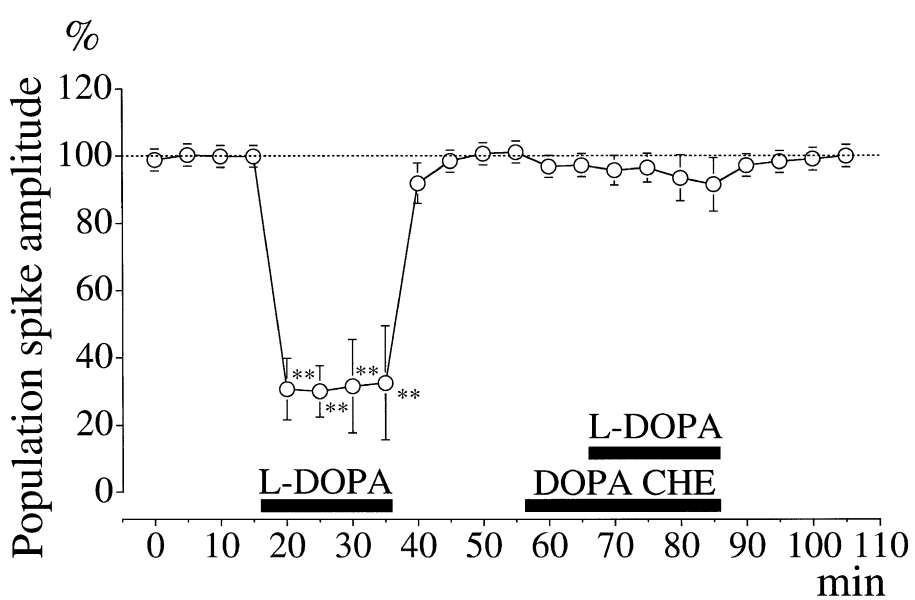

Fig. 4. Antagonistic effects of L-DOPA cyclohexyl ester (DOPA CHE: 30 nM) on L-DOPA (100 nM)-induced inhibition of population spikes. Symbols and bars represent the means and S.E.M., respectively ($n = 5$). L-DOPA and DOPA CHE were applied during the periods indicated by the horizontal bars in the graph. $^{**}P < 0.01$, significantly different from the value before drug application.

Table 1
Effects of phentolamine and bicuculline on L-DOPA-, muscimol-, or 6-fluoronorepinephrine-induced inhibition of population spikes induced by Schaffer collateral/commissural fiber stimulation in the hippocampal CA1 area

	% Changes of population spikes after treatment			
	n	5 min	10 min	15 min
L-DOPA (1 μM)	3			
Alone		26.5 ± 7.4[a,b]	15.4 ± 11.7[a,b]	19.4 ± 15.8[a,b]
+ Phentolamine (10 μM)		112.0 ± 8.8[b]	112.5 ± 10.0[b]	113.7 ± 9.8[b]
Washing		60.8 ± 2.9[a]	18.5 ± 6.0[a]	19.2 ± 7.0[a]
L-DOPA (1 μM)	6			
Alone		38.0 ± 13.4[a,b]	38.1 ± 12.2[b,c]	46.7 ± 14.3[c,d]
+ Bicuculline (1 μM)		105.8 ± 12.3[b]	108.1 ± 16.3[b]	112.5 ± 17.5[d]
Washing		56.0 ± 12.8	39.6 ± 13.5[c]	35.4 ± 13.8[c]
Muscimol (1 μM)	5			
Alone		50.6 ± 14.5[c]	56.3 ± 7.3[c]	55.2 ± 12.0[c]
+ Phentolamine (10 μM)		47.8 ± 13.6[c]	45.0 ± 10.2[a]	45.8 ± 12.0[c]
Washing		41.6 ± 12.8[c]	35.3 ± 13.6[a]	41.0 ± 13.6[a]
6-Fluoronorepinephrine (10 μM)	4			
Alone		26.2 ± 11.5[a,b]	19.4 ± 13.6[a,d]	22.4 ± 15.3[a,d]
+ Bicuculline (1 μM)		77.0 ± 8.8[b]	80.2 ± 14.2[d]	80.3 ± 13.4[d]
Washing		59.8 ± 10.7[c]	44.3 ± 15.2[c]	52.6 ± 14.4

Alone: L-DOPA, muscimol or 6-fluoronorepinephrine alone was applied.
+ Phentolamine or + bicuculline: L-DOPA, muscimol or 6-fluoronorepinephrine was applied simultaneously with phentolamine or bicuculline.
Washing: L-DOPA, muscimol or 6-fluoronorepinephrine alone was applied 20 min after the concomitant application of L-DOPA with phentolamine or bicuculline was stopped.
% Changes: The height of the population spike before application of drugs was taken as 100%.
[a] $P < 0.01$, significantly different from the value before the drug application.
[b] $P < 0.01$, significantly different from the respective value after corresponding treatment with L-DOPA or 6-fluoronorepinephrine alone.
[c] $P < 0.05$, significantly different from the value before the drug application.
[d] $P < 0.05$, significantly different from the respective value after corresponding treatment with L-DOPA or 6-fluoronorepinephrine alone.

after application of L-DOPA (1 μM) in the presence of bicuculline (1 μM) (Table 1). Inhibition of the population spike was also observed with 6-fluoronorepinephrine (10 μM), an α-adrenoceptor agonist. The 6-fluoronorepinephrine-induced inhibition was antagonized by bicuculline (1 μM). The population spike amplitude, which was reduced to 22.4 ± 15.3% ($n = 4$) 15 min after application of 6-fluoronorepinephrine (10 μM), was significantly ($P < 0.05$) increased to 80.3 ± 13.4% 15 min after simultaneous application of bicuculline (1 μM) (Table 1). In contrast, the muscimol (1 μM)-induced inhibition of the population spike amplitude was not affected by phentolamine (10 μM).

4. Discussion

L-DOPA at concentrations as low as 1 nM was found to inhibit the population spike induced by stimulation of Schaffer collateral/commissural fibers in the CA1 area of hippocampal slices. This potency of L-DOPA has also been observed in the hypothalamus (Goshima et al., 1991b). The L-DOPA-induced inhibition was unaffected by the presence of NSD-1015, an aromatic amino acid decarboxylase inhibitor. Therefore, L-DOPA-induced inhibition was considered to be due to L-DOPA itself and not to dopamine produced from L-DOPA by aromatic amino acid decarboxylase, as reported in the hypothalamus and striatum (Goshima et al., 1988, 1990b; Nakamura et al., 1992). Furthermore, L-DOPA-induced inhibition was blocked by the presence of L-DOPA cyclohexyl ester, which is a reversible and competitive antagonist of L-DOPA recognition sites (Furukawa et al., 2000; Misu et al., 1997). Another L-DOPA recognition site antagonist, L-DOPA methylester, has been found to antagonize L-DOPA-induced noradrenaline release from the hypothalamus (Goshima et al., 1991a). Together with these previous findings, the present findings suggest that the effects of L-DOPA are mediated by putative L-DOPA recognition sites.

It was noteworthy that the L-DOPA-induced inhibition of the population spike in the CA1 area was antagonized by phentolamine, an α-adrenoceptor antagonist. These results suggest that L-DOPA acts on the putative L-DOPA recognition sites located on noradrenergic nerve terminals, thereby releasing noradrenaline to inhibit the population spikes in the CA1 areas. This conclusion is supported by the findings that 6-fluoronorepinephrine, an α-adrenoceptor agonist, inhibited the population spike in this area. Actually, L-DOPA has been reported to release noradrenaline in hypothalamic slices (Sato et al., 1993). Moreover, the L-DOPA-induced inhibition of the population spike was antagonized by bicuculline, a $GABA_A$ receptor antagonist, and was mimicked by muscimol, a $GABA_A$ receptor

agonist. Interestingly, the inhibitory effect of 6-fluoronorepinephrine on the population spike was also blocked by bicuculline. These findings indicate that L-DOPA increases noradrenaline release, which in turn releases GABA, which inhibits the population spikes by acting on the $GABA_A$ receptor. However, the muscimol-induced inhibition was not affected by phentolamine. Therefore, the muscimol-induced inhibition of the population spike might not affect noradrenergic transmission in the hippocampus via α-adrenoceptors.

In addition to noradrenaline, glutamate and dopamine are released by L-DOPA in hypothalamic and striatal slices (Goshima et al., 1986, 1991a, 1993; Maeda et al., 1997; Snyder and Zigmond, 1990). Therefore, the increases in population spikes observed in the present study during simultaneous application of L-DOPA with phentolamine or bicuculline may have been at least partly due to an enhanced release of glutamate as a result of blockade of GABA-induced inhibitory effects. Furthermore, the decrease in L-DOPA-induced inhibition at high concentrations of the drug may have been due to a dominant increase (micromolar order) in glutamate release, since noradrenaline release was induced at much lower concentrations (picomolar order) (Goshima et al., 1986, 1991b, 1993).

In conclusion, L-DOPA is suggested to act on the putative L-DOPA recognition sites located on noradrenergic nerve terminals derived from the locus coeruleus to release noradrenaline, which then releases GABA by acting on the α-adrenoceptors in GABA-containing cells and/or their nerve terminals, thereby inhibiting CA1 neurons via $GABA_A$ receptors.

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