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Presynaptic modulation of synaptic γ-aminobutyric acid transmission by tandospirone in rat basolateral amygdala

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Abstract

Nystatin-perforated patch recordings were made from mechanically dissociated neurons (in which functional native presynaptic nerve terminals are preserved), isolated from the basolateral amygdala regions to investigate the effects of tandospirone on γ-aminobutyric acidergic (GABAergic) inhibition. Two types of neurons, ovoid-shaped and pyramidal-shaped neurons, were obtained from the basolateral amygdala nuclei and the electrophysiological characteristics of these two types of neurons supported the morphological classification of these isolated neurons. From the ovoid-shaped neurons, bicuculline-sensitive $GABA_A$ergic miniature inhibitory postsynaptic currents (miniature IPSC) were recorded in the presence of tetrodotoxin, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and DL-2-amino-5-phosphovaleric acid (DL-AP5). Tandospirone (10 μM) reversibly and continuously inhibited the GABAergic miniature synaptic events to 66.3 ± 2.1% of control ($P < 0.01$, $n = 17$) without affecting the miniature IPSC amplitude (104.0 ± 3.1% of control, $n = 17$). The similar inhibition of miniature IPSC frequency was mimicked by a specific 5-HT_{1A} receptor agonist 8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT, 1 μM), and the effects of tandospirone were prevented in the presence of a specific 5-HT_{1A} receptor antagonist 1-(2-methoxyphenyl)-4-[4-(2-phthalimido)butyl] piperazine hydrobromide (NAN-190, 1 μM). Activation of 5-HT_{1A} receptors by 8-OH-DPAT (1 μM) evoked no direct postsynaptic effects in enzyme-treated isolated basolateral amygdala neurons, suggesting that tandospirone acts at presynaptic 5-HT_{1A} receptors. Furthermore, this presynaptic inhibition by tandospirone was prevented after treatment with a pertussis toxin-sensitive GTP-binding protein (G-protein) inhibitor, *N*-ethylmaleimide (at 3 μM for 5 min). In conclusion, in the basolateral amygdala nuclei, tandospirone activated presynaptic 5-HT_{1A} receptors on the GABAergic nerve terminals projecting to ovoid-shaped neurons and inhibited synaptic GABA transmission via G-proteins.

Keywords: Basolateral amygdala; Synaptic γ-aminobutyric acid (GABA) transmission; 5-HT_{1A} receptors; Presynaptic modulation; Dissociated neuron and tandospirone

1. Introduction

Tandospirone, a pyrimidylpiperazine derivative and a 5-HT_{1A} receptor agonist, is widely prescribed as a potent anxiolytic agent (Shimizu et al., 1988; Hamik et al., 1990). In animal models, the '5-HT behavioral syndrome', which includes hyperlocomotion, head weaving, a flat body posture and reciprocal forepaw treading (Tricklebank et al., 1985), was blocked by systemic application of tandospirone, consistent with its anti-anxiety effects (Wieland et al., 1993). The anxiolytic mechanism of tandospirone is so far considered to depend on the inhibition of serotonergic neurons in the raphe nuclei. Tandospirone activates 5-HT_{1A} receptors located on proximal dendrites or soma and opens potassium channels, thus inhibiting the excitability of serotonin-containing neurons by causing membrane hyperpolarization. This leads to a decrease in serotonin released to various targets in the brain (Haj-Dahmane et al., 1991; Jin and Akaike, 1998). However, since systemically administered tandospirone can reach the amygdala nuclei, which are significant for the regulation of anxiety and have abundant 5-HT_{1A} receptor expression (Marcinkiewicz et al., 1984), tandospirone also effectively affects the amygdala nuclei, exhibiting anxiolytic effects. The amygdala nuclei are further divided into functional

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units such as olfactory, autonomic and frontotemporal systems and, among them, the frontotemporal system is physiologically significant for anxiety-related behaviors in animals (Swanson and Petrovich, 1998). In the frontotemporal system, basolateral amygdala nuclei have been extensively investigated in anatomical and histological studies to establish the classification of cell types in the nucleus and the neuronal connections with other brain regions (Millhouse and DeOlmos, 1983; Sripanidkulchai et al., 1984; McDonald, 1985, 1996). Considering these previous findings, we investigated where and how tandospirone acts in the amygdala nuclei to contribute to anxiolysis. γ-aminobutyric acidergic (GABAergic) interneurons play a critical role in the regulation of excitatory outputs from basolateral amygdala nuclei to prefrontal areas in the cortex (Pare and Gaudreau, 1996). In addition, we previously showed that activation of presynaptic 5-HT_{1A} receptors inhibits synaptic GABA release from attached basolateral amygdala neurons (Koyama et al., 1999). Therefore, we hypothesized that tandospirone modulates synaptic GABA transmission via presynaptic 5-HT_{1A} receptors regulating the membrane excitability of interneurons and thus, possibly contributes to anxiolysis. To examine this hypothesis, we first classified mechanically dissociated basolateral amygdala neurons according to their shape and electrophysiological characteristics. Second, we checked whether tandospirone has a direct postsynaptic effect via 5-HT_{1A} receptors. Finally, we quantitatively examined presynaptic modulation by tandospirone.

2. Materials and methods

2.1. Preparations

Experimental protocols were approved by the Ethics Review Committee for Animal Experimentation of our institution. Wistar rats (10–14-days old) were decapitated under pentobarbital anesthesia (50 mg/kg i.p.). The brains were quickly removed and transversely sliced at a thickness of 500 μm (DM IRB, Leica, Germany). Slices were incubated in control medium (see below) saturated with 95% O_2 and 5% CO_2 at room temperature (20–22°C) for at least 1 h. For dissociation, slices were transferred into a 35-mm culture dish (Primaria 3801, Becton Dickinson, NJ, USA) and the basolateral region of the amygdala was identified under a binocular microscope (SMZ-1, Nikon, Tokyo, Japan). Neurons were then mechanically dissociated using a vibrating style that yielded neurons with attached native presynaptic nerve terminals (Koyama et al., 1999). To examine the direct postsynaptic effects of 5-HT_{1A} receptor agonists, we isolated neurons by treatment with enzymes. Transverse brain slices at a thickness of 400 μm were preincubated in the control medium for 1 h and then treated with 0.3 mg/ml dispase at 31°C for 40–50 min. The basolateral regions were identified and then removed with a micropunch. This micropunch sample was dissociated by mild trituration, using fire-polished Pasteur pipettes, in the 35-mm culture dish. Individual neurons settled and adhered to the bottom of the dish over 20 min following dispersion.

2.2. Electrical measurement

All electrical measurements were performed using the nystatin-perforated patch recording method, allowing the intracellular signaling system to remain as intact as possible (Akaike and Harata, 1994). All voltage-clamp recordings were made at a holding potential of −70 mV (CEZ-2300, Nihon Kohden, Tokyo, Japan). Patch pipettes were pulled from borosilicate capillary glass (1.5 mm o.d., 0.9 mm i.d.; G-1.5, Narishige, Tokyo, Japan) in two stages on a vertical pipette puller (PB-7, Narishige). The resistance of the recording electrode filled with pipette solution was 5–7 MΩ. Neurons were visualized under phase-contrast on an inverted microscope (Diapot, Nikon). Current and voltage were continuously monitored on an oscilloscope (Textronix 5111A, Sony, Tokyo, Japan) and recorded on a paper chart recorder (Recti-Horiz 8K, Nippondenki San-ei, Tokyo, Japan) as well as on videotape (PCM-501 ES, Sony). Membrane currents were filtered at 1 kHz (E-3201A Decade Filter, NF Electronic Instruments, Tokyo, Japan) and digitized at 4 kHz. All experiments were performed at room temperature (20–22°C).

2.3. Data analysis

Miniature inhibitory postsynaptic currents (miniature IPSC) were collected in pre-set epochs, before, during and after each experimental condition. Inclusion criterion required a minimum event duration of 1 ms. Current events were counted and analyzed using DETECTiVENT software (Ankri et al., 1994) and IGOR pro software (Wavemetrics, Lake Oswego, OR, USA). Numerical data are presented as the means ± S.E.M. Differences in amplitude and frequency distributions of miniature IPSCs were checked by non-parametric analysis (Kolmogorov–Smirnov test) with a significant difference of $P < 0.05$. Measured mean amplitude and event number of miniature IPSCs were normalized to those of control and calculated by Student's two-tailed *t*-test. All statistical analyses were performed using StatView software (version J-4.5).

2.4. Solutions

The ionic composition of the incubation medium was (in mM): 124 NaCl, 5 KCl, 1.2 KH_2PO_4, 24 $NaHCO_3$, 2.4 $CaCl_2$, 1.3 $MgSO_4$ and 10 glucose bubbled with 95%O_2

and 5%CO_2. The standard external solution was (in mM): 150 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 glucose and 10 HEPES. The pH of the external solution was adjusted to 7.4 with tris(hydroxymethyl)aminomethane (Tris-OH). In order to isolate spontaneous miniature IPSCs, external solutions routinely contained 300 nM tetrodotoxin to block voltage-dependent Na^+ channels, and 1 μM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and 10 μM DL-2-amino-5-phosphovaleric acid (DL-AP5) to eliminate glutamatergic components. The ionic composition of the internal (patch-pipette) solution for the nystatin-perforated recordings was (in mM): 20 *N*-methyl-D-glucamine methanesulfonate, 20 Cs-methanesulfonate, 5 $MgCl_2$, 100 CsCl and 10 HEPES. To examine postsynaptic effects of 5-HT_{1A} receptors, Cs-methanesulfonate and CsCl in the upper pipette solution were exchanged for 20 mM K-methanesulfonate and 100 mM KCl, respectively. For nystatin-perforated current clamp recordings, the patch pipette contained 150 mM KCl and 10 mM HEPES. The pH of these internal solutions was adjusted to 7.2 with Tris-OH. Nystatin was dissolved in acidified methanol at 10 mg/ml. This stock solution was diluted with the internal solution just before use to a final concentration of 100–200 μg/ml.

2.5. Drugs

Drugs used in the present study included DL-AP5, bicuculline, CNQX, *N*-ethylmaleimide and nystatin from Sigma (USA). Tandospirone was from Sumitomo (Tokyo, Japan). 8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT) and 1-(2-methoxyphenyl)-4-[4-(2-phthalimido)butyl] piperazine hydrobromide (NAN-190) were from Research Biochemicals (USA). Tetrodotoxin was from Wako (Tokyo, Japan). Dispase I was from Boehringer-Mannheim (Germany). Drugs were applied by our 'Y-tube system' for rapid solution exchange (Murase et al., 1990).

3. Results

3.1. Morphological and electrophysiological properties of basolateral amygdala neurons

After mechanical dissociation, two cell types were distinguished on the basis of neuronal shape, namely, pyramidal (Fig. 1A.a) and ovoid-shaped neurons (Fig. 1B.a),

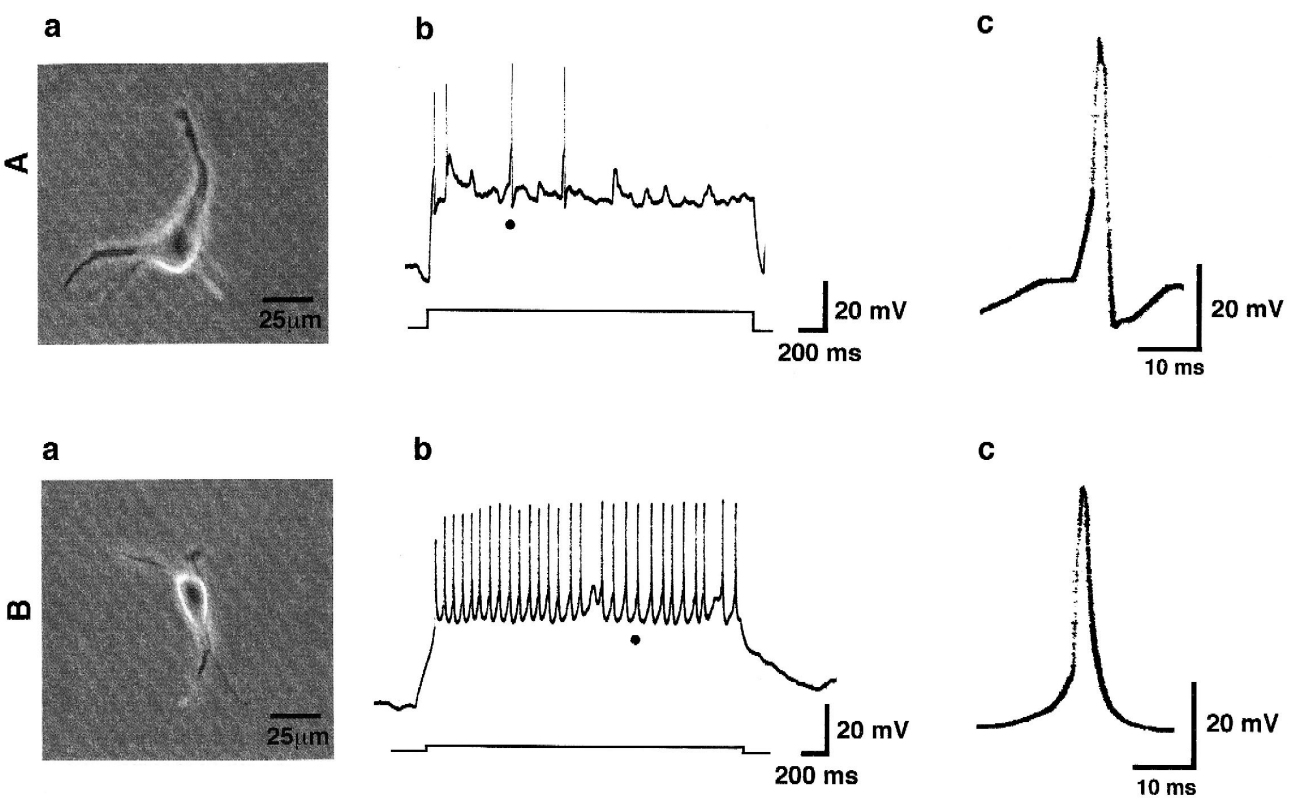

Fig. 1. Morphological and electrophysiological properties of the mechanically dissociated basolateral amygdala neurons. (A.a) Mechanically dissociated pyramidal-shaped neuron. Inset bar is 25 μm. (A.b) Responses to a prolonged current injection of 3.0 nA (2500 ms in duration). (A.c). Single representative action potential from the left trace (black point). (B.a) Mechanically dissociated ovoid-shaped neuron. Inset bar is 25 μm. (B.b) Responses to a prolonged current injection of 1.0 nA (2500 ms in duration). (B.c) Single representative action potential from the left trace (black point). Resting membrane potential was −70 mV.

consistent with previous studies (Millhouse and DeOlmos, 1983; McDonald, 1985). The original properties of the neurons, including short portions of their proximal dendrites, were well preserved. Functionally, two types of neurons were encountered, based on their electrophysiological characteristics, which supports the findings of previous studies of slice preparations from this area (Washburn and Moises, 1992; Rainnie et al., 1993; Mahanty and Sah, 1998). In 5 out of 10 pyramidal-shaped neurons, prolonged current injections evoked limited action potentials, exhibiting spike frequency adaptation (Fig. 1A.b). In contrast, a train of action potentials was observed without any spike frequency adaptation in five out of seven ovoid-shaped neurons (Fig. 1B.b). For a single representative action potential, the after-hyperpolarization was pronounced in the pyramidal-shaped neurons, but was shallow in the ovoid-shaped neurons (Fig. 1A.c and B.c). These results suggest that the mechanically dissociated basolateral amygdala neurons could be divided into pyramidal and ovoid-shaped neurons depending on their electrophysiological properties. We selected the ovoid-shaped neurons for the following experiments, since interneurons are suggested to be critical in the regulation of excitatory outputs from the basolateral nuclei to prefrontal cortex areas (Pare and Gaudreau, 1996).

3.2. GABAergic miniature IPSCs

In the presence of tetrodotoxin (300 nM), CNQX (1 μM) and DL-AP5 (10 μM), inward miniature IPSCs were

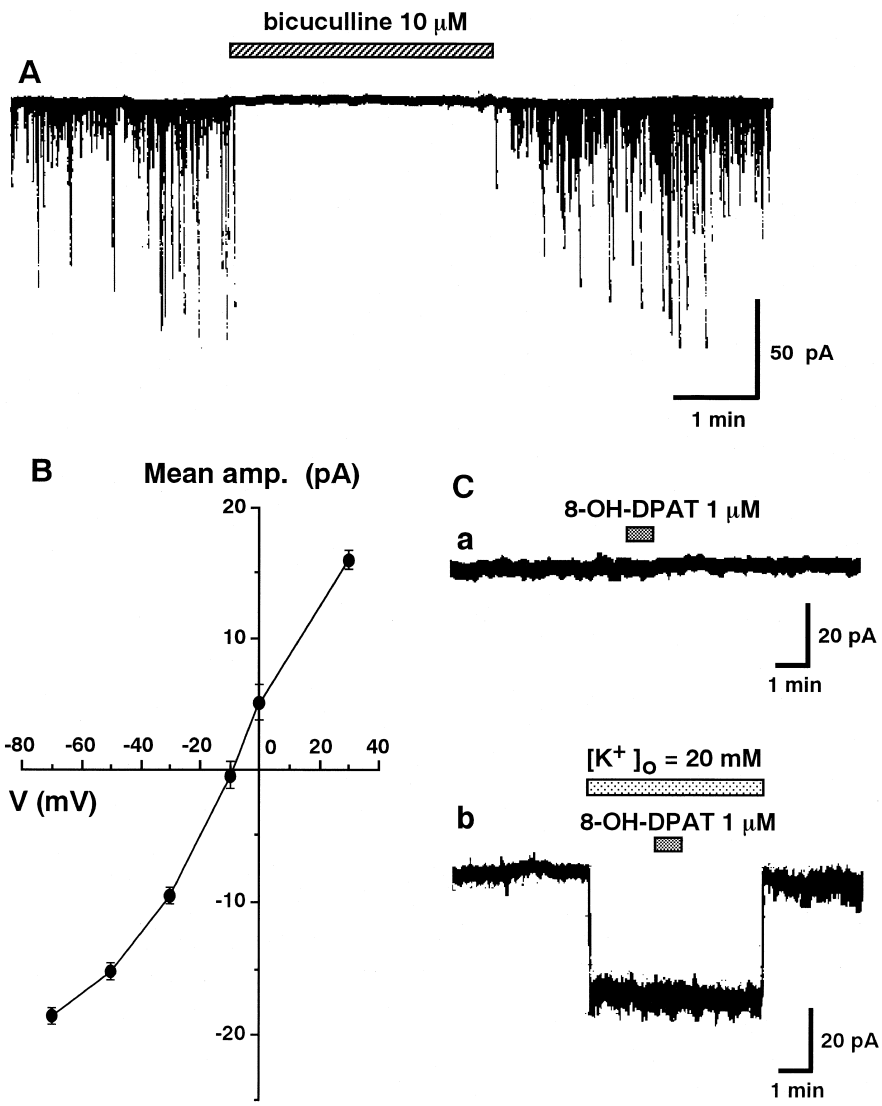

Fig. 2. $GABA_A$ergic miniature inhibitory postsynaptic currents. (A) Complete blockade of miniature IPSCs by bicuculline in the presence of tetrodotoxin, CNQX and DL-AP5. (B) Relationship for voltage and the mean amplitude of miniature IPSCs. The mean amplitude was obtained from analysis of the miniature events over 5 min. Each point is the mean for five neurons. The vertical bar shows ±S.E.M. (C) No direct postsynaptic effects evoked by 8-OH-DPAT in normal and high concentration of extracellular potassium.

recorded at a holding potential of −70 mV. Bicuculline (10 μM), a specific $GABA_A$ receptor antagonist, completely and reversibly blocked the miniature IPSCs (Fig. 2A). When miniature IPSCs were recorded at various holding potentials, the *I–V* relation for these miniature IPSCs exhibited outward rectification and reversed at -10.7 ± 1.8 mV ($n = 5$) (Fig. 2B). This reversal potential is quite similar to the theoretical Cl^- equilibrium potential obtained with the external and internal solutions containing 161 and 110 Cl^-, respectively (E_{Cl}: −9.6 mV). These results suggest that the miniature IPSCs were $GABA_A$ receptor-mediated Cl^- currents.

Next, we checked possible postsynaptic effects of 5 HT_{1A} receptor agonists by using dispase-treated basolateral ovoid shaped neurons, since activation of postsynaptic 5 HT_{1A} receptors opens inward rectifying K^+ channels (Jin and Akaike, 1998). At a holding potential of −70 mV, a specific 5 HT_{1A} receptor agonist, 8-OH-DPAT (1 μM), did not evoke a postsynaptic response (Fig. 2C.a). Then, we increased the extracellular K^+ concentration from 5 to 20 mM and applied 8-OH-DPAT. However, no postsynaptic response could be detected (Fig. 2C.b). Similar results were obtained for 20 other neurons, which suggests that the ovoid-shaped neurons lack postsynaptic responses via 5-HT_{1A} receptors.

3.3. Effects of tandospirone on miniature IPSCs

We investigated the effects of tandospirone on the GABAergic miniature IPSCs. Tandospirone (10 μM) potently and consistently inhibited miniature IPSC frequency and this effect was reversible (Fig. 3A, left). The time course of the miniature synaptic events before, during and after the application of tandospirone was plotted (Fig. 3B). Tandospirone continuously decreased the number of miniature synaptic events. Analysis using the cumulative probability plots from the upper trace in Fig. 3A (left) showed that tandospirone had no effect on the amplitude distribution of miniature IPSCs (Kolmogorov–Smirnov test, $P = 2.0$, numbers of events 421 for control and 325 for tandospirone) (Fig. 3C). From 17 neurons examined, tandospirone dose dependently decreased the miniature IPSC frequency ($P < 0.01$) without affecting the miniature IPSC

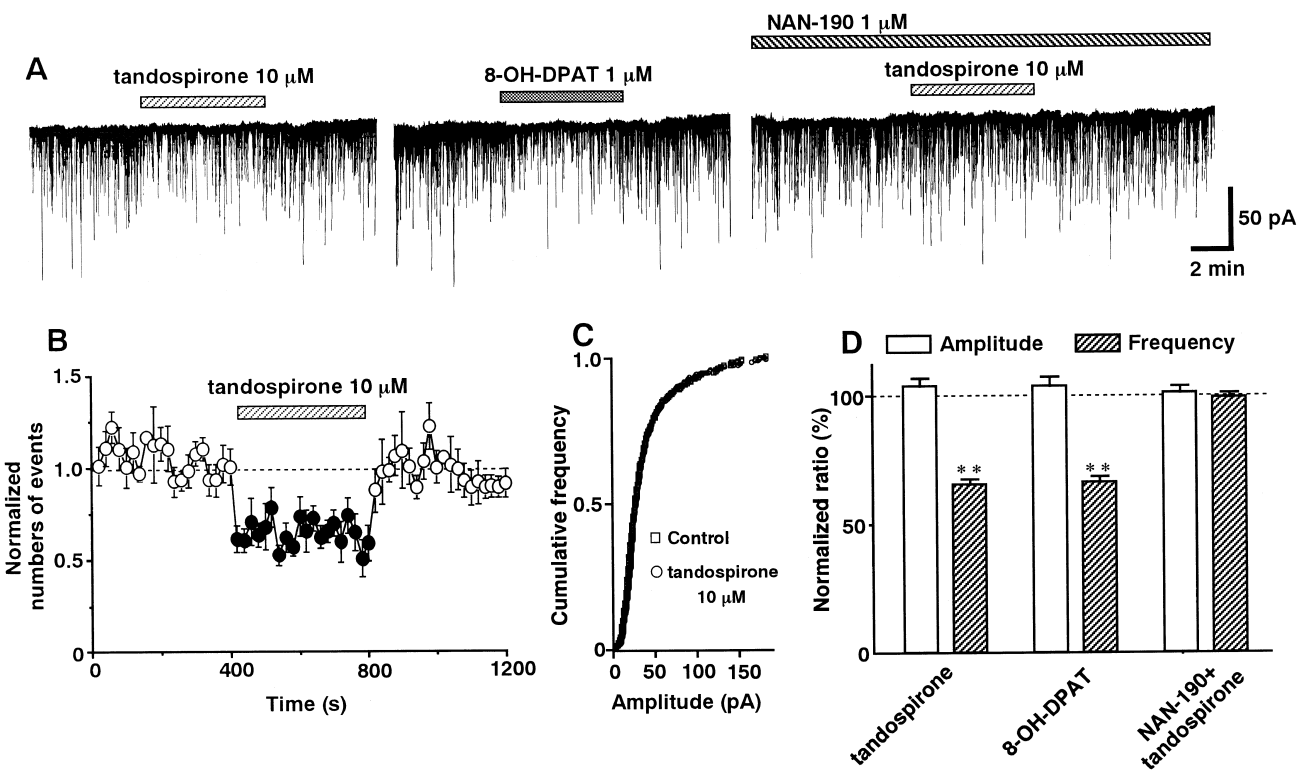

Fig. 3. Effects of tandospirone on the miniature IPSCs. (A) Recordings of 5-HT_{1A} receptor agonists, tandospirone and 8-OH-DPAT and antagonist, NAN-190 from the same neuron. (B) Time course of miniature synaptic events before, during and after the application of tandospirone. Events recorded every 10 s were normalized to the averaged synaptic event every 10 s from before and after controls. Each point is the mean for seven neurons. The vertical bar shows ±S.E.M. (C) The cumulative probability plots of the mean amplitude of the miniature IPSCs in control and tandospirone-treated neurons. (D) All amplitudes and frequencies were normalized to those of control. *Amplitude* (percentage of control): tandospirone, 104.0 ± 3.1, $n = 17$; 8-OH-DPAT, 104.3 ± 3.6, $n = 16$; NAN-190 + tandospirone, 101.0 ± 3.0, $n = 10$. *Frequency* (percentage of control): tandospirone, 66.3 ± 2.1, $n = 17$; 8-OH-DPAT, 66.0 ± 2.2, $n = 16$; NAN-190 + tandospirone, 100.5 ± 1.8, $n = 10$. The vertical bar shows ±S.E.M. Asterisks represent statistically significant differences ($^{**}P < 0.01$, Student's two-tailed *t*-test).

Table 1
Effects 5-HT_{1A} receptor agonists on miniature IPSCs

Concentration (M)	Tandospirone			8-OH-DPAT		
	Amplitude	Frequency	*n*	Amplitude	Frequency	*n*
10^{-9}	–	–	–	112 ± 2.8	100 ± 8.6	4
10^{-8}	100 ± 5.9	99 ± 4.3	4	105 ± 2.7	112 ± 3.1	4
10^{-7}	102 ± 2.8	99 ± 1.0	4	103 ± 5.2	84 ± 3.8	4
10^{-6}	96 ± 2.0	84 ± 4.9	6	104 ± 3.6	66 ± 2.2[a]	16
10^{-5}	104 ± 3.1	66 ± 2.1[a]	17	–	–	–

The amplitude and frequency of miniature IPSCs were normalized to those of control. The vertical bar shows ±S.E.M. Asterisks represent statistically significant differences.
[a] $P < 0.01$; Student's two-tailed *t*-test.

amplitude (Fig. 3D and Table 1). 8-OH-DPAT (1 μM) mimicked these effects of tandospirone on miniature IPSCs (Fig. 3A, middle and D). 8-OH-DPAT also caused a dose-dependent inhibition but inhibited the miniature IPSC frequency with 10 times the potency of tandospirone (Table 1). A specific 5-HT_{1A} receptor antagonist, NAN-190 (1 μM), itself affected neither the miniature IPSC amplitude (101.0 ± 2.3% of control, $n = 10$) nor the miniature IPSC frequency (100.0 ± 0.9% of control, $n = 10$). However, NAN-190 (1 μM) prevented the inhibition of the miniature IPSC frequency evoked by tandospirone (Fig. 3A, right and D). All these findings and the lack of a direct postsynaptic response via 5-HT_{1A} receptors (Fig. 2C) suggest that tandospirone activated presynaptic 5-HT_{1A} receptors to inhibit synaptic GABA transmission in nerve terminals.

3.4. Effects of N-ethylmaleimide

In our previous study, presynaptic 5-HT_{1A} receptors were found to be coupled to GTP-binding protein (G-pro-

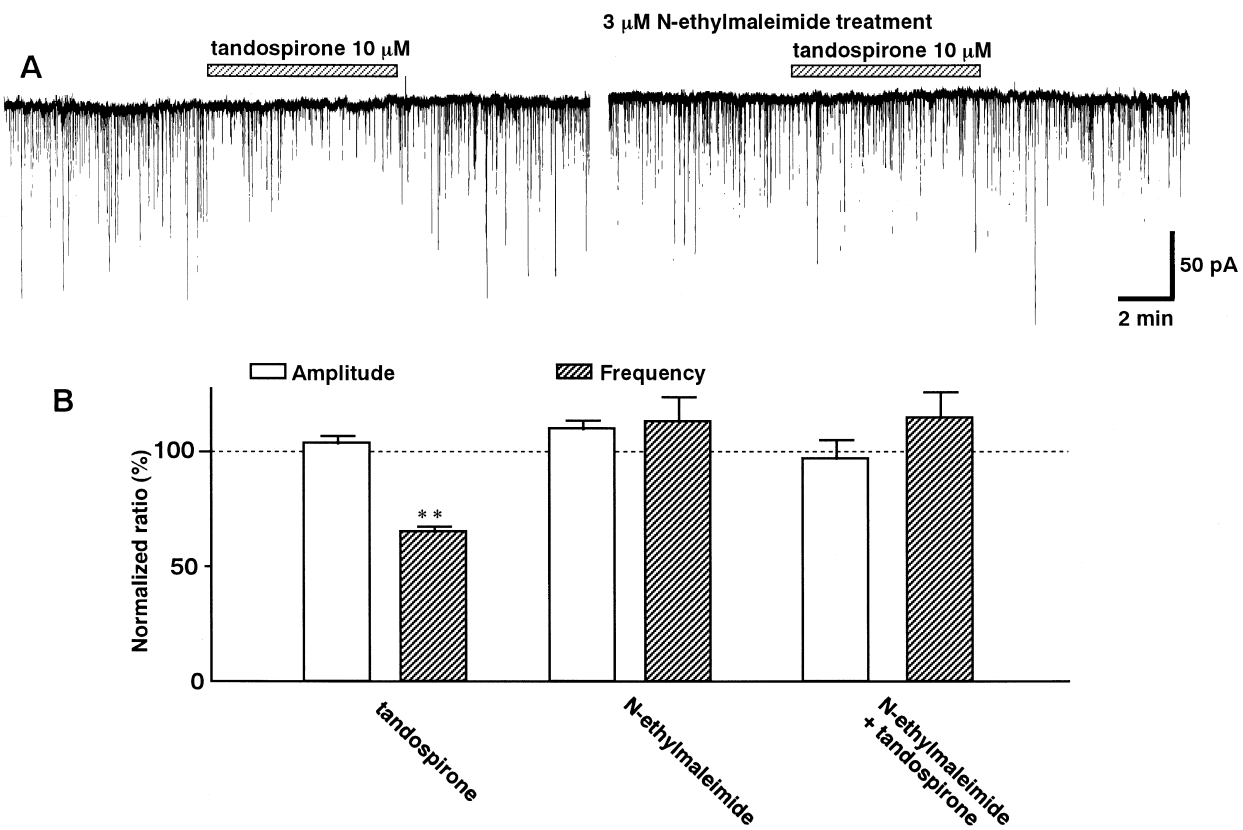

Fig. 4. Effects of *N*-ethylmaleimide. (A) Recordings of the effect of tandospirone before and after treatment with *N*-ethylmaleimide in the same neuron. (B) All amplitudes and frequencies were normalized to those of control. *Amplitude* (percentage of control): tandospirone, 103.7 ± 2.8, $n = 5$; *N*-ethylmaleimide, 110.0 ± 3.6, $n = 9$; *N*-ethylmaleimide + tandospirone, 98.4 ± 7.3, $n = 5$. *Frequency* (percentage of control): tandospirone, 67.0 ± 1.8, $n = 5$; *N*-ethylmaleimide, 113.6 ± 11.4, $n = 9$; *N*-ethylmaleimide + tandospirone, 115.8 ± 11.5, $n = 5$. The vertical bar shows ±S.E.M. Asterisks represent statistically significant differences ($^{**}P < 0.01$, Student's two-tailed *t*-test).

tein) in basolateral neurons (Koyama et al., 1999). Therefore, we examined whether tandospirone could activate G-protein-coupled 5-HT_{1A} receptors using the sulphydryl alkylating agent *N*-ethylmaleimide, a pertussis toxin-sensitive G-protein inhibitor (Asano and Ogasawara, 1986). Treatment with *N*-ethylmaleimide at 3 μM for 5 min did not significantly change the miniature IPSC amplitude or the miniature IPSC frequency (Fig. 4B). Before treatment with *N*-ethylmaleimide, tandospirone inhibited the miniature IPSC frequency ($P < 0.01$, $n = 5$) (Fig. 4A, left and B). However, the treatment with *N*-ethylmaleimide completely abolished the inhibition of the miniature IPSC frequency by tandospirone, suggesting that on intracellular signalling pathway via G-proteins participates in the mechanism of presynaptic inhibition by tandospirone (Fig. 4A, right and B).

4. Discussion

In the present study, we mechanically dissociated neurons, to preserve functional presynaptic nerve terminals, from the basolateral amygdala. Two types of neurons, ovoid-shaped and pyramidal-shaped neurons, were observed and their electrophysiological characteristics were also different. From the ovoid-shaped neurons, GABAergic miniature IPSCs were recorded. Tandospirone inhibited synaptic GABA release via G-protein-coupled presynaptic 5-HT_{1A} receptors located on GABAergic nerve terminals projecting to the ovoid-shaped neurons.

4.1. Anxiolytic effects of tandospirone via 5-HT_{1A} receptors on the GABAergic nerve terminals

Serotonin-containing neurons are concentrated in the raphe nuclei and have widespread projections in the brain. So far, it is considered that tandospirone directly inhibits these serotonergic neurons and subsequently decreases serotonin release in the territories of projection, exhibiting anxiolytic effects (Haj-Dahmane et al., 1991; Jin and Akaike, 1998). Therefore, the anxiolysis evoked by tandospirone depends on the 5-HT_{1A} receptor functioning as an autoreceptor in a negative feedback manner. In the present study, we showed that the ovoid-shaped neurons lacked postsynaptic responses via 5-HT_{1A} receptors and that tandospirone activated presynaptic 5-HT_{1A} receptors on GABAergic nerve terminals attached to the ovoid-shaped neurons, thus exhibiting completely different mechanisms in the raphe nuclei (Figs. 2C and 3). While our study has the limitation that dissociated basolateral amygdala neurons were identified only by their shape and electrophysiological characteristics, our results were consistent with those of previous studies (Washburn and Moises, 1992; Rainnie et al., 1993; Mahanty and Sah, 1998). Considering this classification of neurons within the amygdala and the neuronal connections between the basolateral amygdala nuclei and frontotemporal regions in the cortex, we can determine how tandospirone contributes to anxiolysis via the basolateral amygdala nuclei. Non-pyramidal ovoid-shaped interneurons contain GABA as a neurotransmitter and receive GABAergic inputs from local other non-pyramidal neurons (Le Gal La Salle et al., 1978; Nitecka and Ben-Ari, 1987). In contrast, pyramidal neurons contain excitatory amino acids such as glutamate or aspartate and project their axons to prefrontal areas in the cortex, where cognitive–behavioral information is organized (Sripanidkulchai et al., 1984; McDonald, 1996). In the amygdala, the non-pyramidal neurons make axosomatic or axo-dendritic synapses with the pyramidal neurons and regulate the excitability of the pyramidal neurons (McDonald, 1985; Pare and Gaudreau, 1996; Mahanty and Sah, 1998). Therefore, inhibition of synaptic GABA release onto the ovoid-shaped neurons by tandospirone disinhibits the ovoid-shaped neurons, consequently increasing GABA release onto the pyramidal neurons. The pyramidal neurons are then inhibited, followed by a subsequent decrease in excitatory amino acid release in the prefrontal cortex.

It is uncertain which subtypes of 5-HT_{1A} receptors exist on presynaptic nerve terminals and are involved in the regulation of neurotransmission. Immunohistochemical study suggests that 5-HT_{1A} receptors are expressed on somato-dendritic regions and that 5-HT_{1B} receptors are expressed on presynaptic nerve terminals (Riad et al., 2000). Electrophysiological study of the basolateral amygdala nuclei suggests that 5-HT_{1a} receptors do not participate in the regulation of neurotransmission (Rainnie, 1999). However, 5-HT_{1A} receptors inhibit synaptic glutamate release in the basolateral nuclei (Cheng et al., 1998) and synaptic GABA release in the dorsal motor nuclei of the vagus (Browning and Travagli, 1999). This discrepancy is partly explained by the wide distribution of serotonin receptors remote from release sites, providing evidence for diffuse serotonin transmission in the central nervous system (Bunin and Wightman, 1999; Riad et al., 2000). Since our preparations were not brain slices but dissociated neurons attached to native synaptic boutons, 5-HT_{1A} receptors are likely to exist on GABAergic nerve terminals regulating synaptic GABA transmission. But it is still possible that both 5-HT_{1A} and 5-HT_{1B} receptors exist on presynaptic nerve terminals (Bobker and Williams, 1989). Our results suggest no direct effects are exerted via postsynaptic 5-HT_{1A} receptors (Fig. 2C), supporting the results of previous studies of the basolateral amygdala neurons (Cheng et al., 1998; Rainnie, 1999). Direct postsynaptic effects of serotonin seem to be evoked via 5-HT_2 receptors (Rainnie, 1999; Browning and Travagli, 1999), implying that different subtypes of serotonin receptors exist at pre- and postsynaptic sites.

4.2. Intracellular mechanism of tandospirone

In the basolateral amygdala nuclei, we suggest that the activation of 5-HT_{1A} receptors on GABAergic nerve terminals inactivates adenylyl cyclase and cAMP signal transduction via G-proteins, thus inhibiting synaptic GABA release, and that the intracellular site of action is downstream to Ca^{2+} influx (Koyama et al., 1999). Therefore, the presynaptic inhibition of synaptic GABA release by tandospirone is considered to depend on the same intracellular mechanism. In contrast, tandospirone activates 5-HT_{1A} receptors on cell bodies in serotonergic neurons, opening inward rectifying K^+ channels (Jin and Akaike, 1998). It is interesting that the pre- and postsynaptic actions of tandospirone use different intracellular signalling pathways, but both seem to contribute to anxiolysis.

4.3. Systemic effects of tandospirone

In the present study, we showed presynaptic modulation by tandospirone and considered its possible anxiolytic mechanism. When tandospirone is applied systemically, the presynaptic action of tandospirone in the amygdala nuclei contributes to anxiolysis, as does its postsynaptic action in the raphe nuclei. Patients suffering from anxiety have variable complaints. The different mechanisms of anxiogenesis in different regions of the brain and the combination of them may give rise to this variability in complaints. Since tandospirone possesses a low affinity for dopamine-2 receptors (Shimizu et al., 1988), possible anxiolytic effects of tandospirone may reflect on additional action on dopamine-2 receptors after systemic application. However, further studies on neuronal connections and areas other than the amygdala and the raphe nuclei in the brain are necessary to understand the precise mechanism of tandospirone in anxiolysis.

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