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Tandospirone-induced K^+ current in acutely dissociated rat dorsal raphe neurones

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1 The effects of tandospirone (TDS) on dissociated rat dorsal raphe neurones were investigated using the patch-clamp method.

2 Under current-clamp conditions, TDS hyperpolarized the cell membrane, resulting in the reduction of firing rates.

3 Under voltage-clamp conditions, TDS induced an inward rectifying K^+ current in a concentration-dependent manner.

4 The TDS-induced K^+ currents (I_{TDS}) were mimicked by 8-OH-DPAT, a 5-HT_{1A} agonist. The I_{TDS} was blocked by spiperone, a 5-HT_{1A} receptor antagonist, in a concentration-dependent manner.

5 N-Ethylmaleimide, an agent which uncouples between the receptor and the G-protein, irreversibly blocked the I_{TDS}.

6 In neurones perfused intracellularly with a pipette-solution containing GTP using the conventional whole-cell patch recording, the I_{TDS} showed a gradual rundown. When the neurones were perfused with GTPγS, TDS activated the inwardly rectifying K^+ current in an irreversible manner.

7 In the inside-out patch recording mode, TDS-activated single K^+ channel currents (i_{TDS}) which also showed an inward rectification. When the GDP in cytosolic side was completely replaced with GTP, the open probability of i_{TDS} significantly increased.

8 These results indicate that the activation of 5-HT_{1A} receptors by TDS directly opens the inward rectifying K^+ channels via a G-protein mediated process.

Keywords: Tandospirone; 5-HT_{1A} receptor; G-protein; inward rectifying K^+ current; signal transduction

Introduction

It is well known that brain 5-hydroxytryptamine (5-HT) receptors mediate various physiological activities (Lucki, 1996). Recent experiments with cloning techniques have revealed that there are more than 10 subtypes of 5-HT receptor (Hoyer *et al*., 1994). Among these 5-HT receptor subtypes, the 5-HT_{1A} receptor is one of the most noteworthy because of its established involvement with psychological disorders. The systemic administration of 5-HT_{1A} receptor agonists causes anxiolytic and antidepressant effects (Blanchard *et al*., 1992; Barf *et al*., 1996; Cervo & Samanin, 1987; Kennett *et al*., 1987). The dorsal raphe (DR) nucleus is a major site of origin for the ascending 5-hydroxytryptaminergic pathways which innervate the hippocampus, neocortex and striatum (Azmitia & Segal, 1978). Moreover, the 5-HT_{1A} receptor plays important roles in the modulation of 5-HT synthesis and release from innervated presynaptic terminals (Bonvento *et al*., 1992). However, the mechanism underlying the anxiolytic activities of the 5-HT_{1A} agonists remains obscure.

Until now, benzodiazepine (BZP) derivatives have usually been used in the treatment of anxiety (Goldberg, 1984; Mennini *et al*., 1987). However, the use of BZP derivatives has been restricted by their relatively severe side effects, such as incoordination, sedation and addiction (Rickels, 1983). Therefore, the use of non-benzodiazepine derivatives, especially those which react with the 5-HT_{1A} receptors of the brain, has recently increased (De Vry, 1995). Tandospirone (TDS), a pyrimidylpiperazine derivative, is one of these 5-HT_{1A} receptor agonists and has been used as an anxiolytic drug (Shimizu *et al*., 1988; Hamik *et al*., 1990). However, the pharmacological mechanism of action for TDS is not well understood. In the present study, the signal transduction mechanism at the receptor-channel level for the novel anxiolytic drug TDS has been investigated in acutely dissociated dorsal raphe (DR) neurones using the patch-clamp technique.

Methods

Preparation

Neurones from the dorsal raphe (DR) nucleus were acutely dissociated from 12–15 day-old Wistar rats of either sex, according to procedures reported elsewhere (Katayama *et al*., 1997) with some modifications. Briefly, the rats were anaesthetized with pentobarbitone and decapitated. The brain was removed and an area containing the dorsal raphe was cut into coronal slices (400 μm) with a microslicer (DTK-10000, D.S.K., Kyoto, Japan). These slices were pre-incubated for 30 min in a medium saturated with 95% O_2 and 5% CO_2 gas at room temperature (21–23°C). Thereafter, the slices were treated with enzymes. The enzyme treatment consisted of the incubation in 1250 PU ml^{-1} of dispase (Godo-Shusei Co. Ltd., Tokyo, Japan) for 50 min at 31°C. Subsequently, a part of the dorsal raphe nucleus was micropunched out with a polished injection needle (ø 0.65 mm). The micropunched pieces were mechanically triturated with fine Pasteur pipettes in a culture dish (Falcon) filled with a physiological solution. After the dissociated neurones had adhered to the bottom of the dish, the electrophysiological recordings were made.

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Solutions

The ionic composition of the incubation medium was (mM): NaCl 124, KCl 5, KH_2PO_4 1.2, $MgSO_4$ 1.3, $CaCl_2$ 2.4, $NaHCO_3$ 24 and glucose 10. The pH of the incubation medium was adjusted to 7.4 with 95% O_2 and 5% CO_2. The ionic composition of the physiological external solution was (mM): NaCl 150, KCl 5, $MgCl_2$ 1, $CaCl_2$ 2, N-2-hydroxyethylpiperazine-N′-2-ethanesulphonic acid (HEPES) 10 and glucose 10. External solutions containing 20 or 50 mM-K^+ were prepared by replacing the NaCl in the physiological external solution with equimolar KCl. The pH of these external solutions was adjusted to 7.4 with tris (hydroxymethyl) aminomethane base (Tris-base). The ionic composition of the internal patch-pipette solution for nystatin perforated patch recordings was (mM): N-methyl-D-glucamine (NMG)-methanesulphonate 20, KCl 20, $MgCl_2$ 5, K-methanesulphonate 100 and HEPES 10. The pH was adjusted to 7.2 with Tris-base. Nystatin (Sigma, St. Louis, MO) was dissolved in methanol (10 mg ml^{-1}), and then this stock solution was diluted with the internal solution just before use at a final nystatin concentration of 200 μg ml^{-1}. The internal solution for the conventional whole-cell patch recording mode (Figure 5) had the following ionic composition (mM): KCl 28, K-methanesulphonate 110, $MgCl_2$ 1, Mg-ATP 5, GTP (or GDPβS or GTPγS) 0.3, EGTA 5; no added $CaCl_2$ and HEPES 10. The pH of these internal solutions was adjusted to 7.2 with Tris-base. The ionic composition of the patch-pipette solution for the single-channel recording in the inside-out patch recording mode was (mM): KCl 140, $CaCl_2$ 2, $MgCl_2$ 1 tetrodotoxin (TTX) 0.3 μM and HEPES 10 (adjusted to pH 7.4 with Tris-base). The ionic composition of the intracellular side solution for the inside-out patch recording mode was (mM): K-methanesulphonate 106, NaCl 40, $MgCl_2$ 1, EGTA 5, Na_2-ATP 2, GTP (or GDP) 0.1 and HEPES 5 (adjusted to pH 7.2 with Tris-base).

Drugs

The chemical agents or drugs used in the present study included: ethylene glycol bis (β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA), TTX, N-ethylmaleimide (NEM) (Sigma), 8-hydroxy-2-(di-*n*-propylamino) tetrain (8-OH-DPAT), ketanserin tartrate (Research Biochemicals, Natick, MA), guanosine 5′-*O*-3-thiotriphosphate trilithium (GTPγS), guanosine 5′-*O*-2-thiodiphosphate trilithium (GTP-βS), guanosine 5′-triphosphate trisodium (GTP) (Wako, Tokyo, Japan), TDS was kindly donated by Sumitomo Pharmaceuticals Co., Ltd. The drugs which were insoluble in water were first dissolved in dimethylsulphoxide (DMSO), and then the stock solution was diluted with the external solution just before use. The final concentrations of DMSO were always less than 0.1%. At these concentrations, DMSO alone had no effect on membrane potential or electrical activity. The other drugs were dissolved in either the external or internal solution just before use.

The drugs were applied with the Y-tube microperfusion system (Murase *et al.*, 1989). With this technique, the external solution surrounding a neurone could be exchanged within 20 ms.

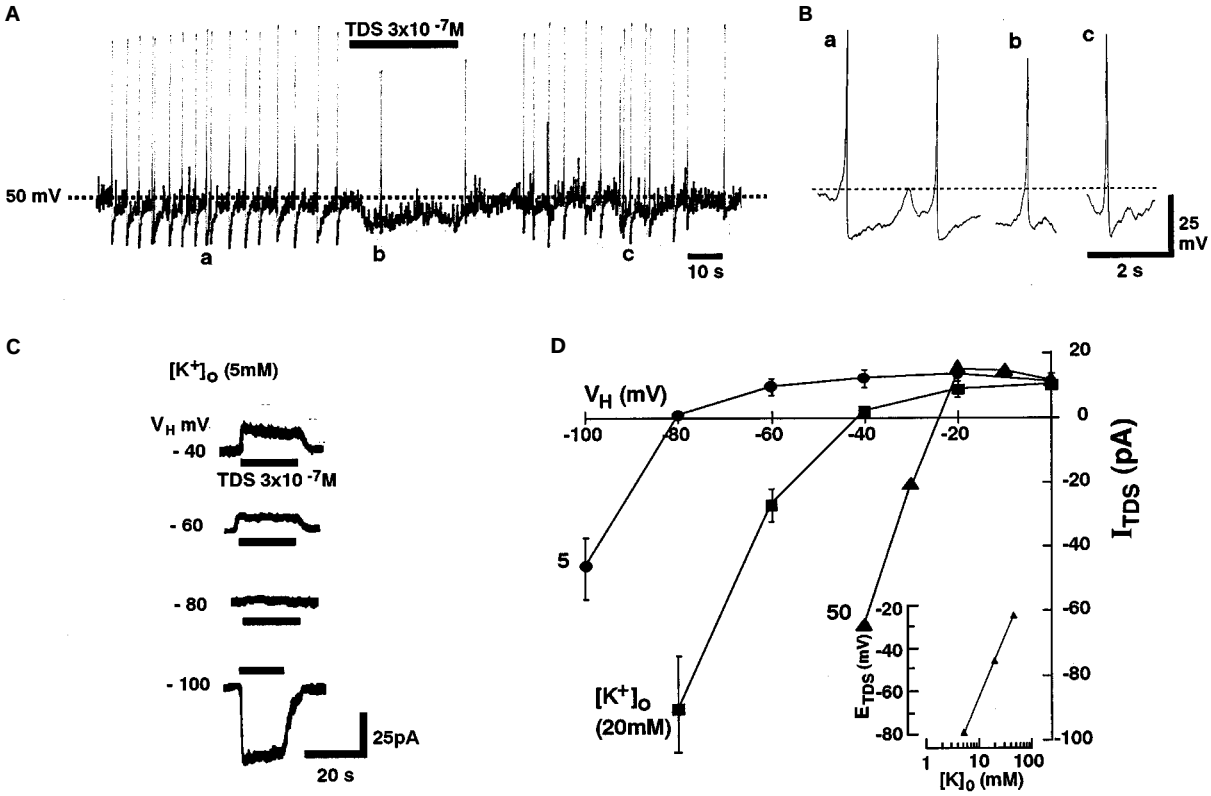

Figure 1 Effect of TDS on dissociated dorsal raphe (DR) neurones. (A) Spontaneous action potentials recorded in the nystatin perforated patch configuration under current-clamp conditions. TDS (3×10^{-7} M) was added to the external solution during a period indicated by the solid line. (B) Digitized potential traces at symbols (a–c) indicated in (A). (C) TDS-induced currents at various holding potentials (V_Hs) in the same neurone perfused with the external solution containing 5 mM K^+. (D) The current-voltage (*I*-V) relationships for the TDS-induced currents in the external solution with three different K^+ concentrations (5, 20 and 50 mM). The intracellular K^+ concentration ($[K^+]_i$) was 120 mM throughout these experiments. Each point and vertical line represent the mean and s.e.mean from 5 neurones. The inset shows the relationship between the external K^+ concentration ($[K^+]_o$) and the reversal potential of TDS-induced current (E_{TDS}). Each E_{TDS} was estimated from the *I*-V relationship.

Electrical measurements

Electrical measurements were performed in the nystatin perforated (Horn & Marty, 1988; Akaike & Harata, 1994), the conventional whole-cell and the inside-out patch recording modes (Hamill *et al*., 1981). The patch pipettes were fabricated from borosilicate glass tubes (G-1.5, Narishige, Tokyo, Japan) using a vertical pipette puller (PB-7, Narishige). The resistance between the recording electrode filled with an internal or external solution and the reference electrode in the normal external solution was 5–7 MΩ. In single-channel experiments, the pipettes were coated with silicone (Shin-Etsu, Tokyo, Japan) near their tips to reduce their capacitance.

The neurones were visualized with a phase-contrast equipment on an inverted microscope (IMT-2, Olympus, Tokyo, Japan). The current and voltage were measured with a patch-clamp amplifier (EPC-7, List Medical, Darmstadt-Eberstadt, Germany), monitored on both a storage oscilloscope (5100A, Iwatsu Eletric, Tokyo, Japan) and a pen recorder (Recti-Horiz-8K, Nipondenki San-ei), and stored on video tapes after digitization with a pulse-coded modulation processor (PCM501 ESN, Nihon Koden, Tokyo Japan).

For single-channel currents, data were filtered using a four-pole low-pass Bessel-type filter (E-3201A, NF Electronic Instruments, Yokohama, Japan) with a -3 dB corner frequency of 2 kHz and sampled every 0.2 ms onto the hard disk of the IBM 386 computer (PS/V entry, Nippon IBM, Tokyo, Japan) using pCLAMP software (version 6.0, Axon Instruments, Inc., Foster, CA). Unitary current amplitudes were measured by forming histograms of baseline and open-level datum points, and fitting these histograms with Gaussian curves using a least-square algorithm to find the area under each curve, and its mean and variance. In the single-channel recordings, the inward currents are shown as downward deflections. The current-voltage (*I*-V) relationships were plotted against the inverted pipette potential (membrane potential, V_m). All experiments were performed at room temperature (21–23°C).

Statistical analysis

Each experimental value is presented as the mean ± s.e.mean. Paired *t* tests were used for statistical analysis. *P* values of less than 0.05 were considered significant. The continuous curves for concentration-response relationships were constructed according to a modified Michaelis-Menten equation (1), using a least-square fitting routine:

$$I = I_{max} \times C^{n_H}/(C^{n_H} + EC_{50}{}^{n_H}) \quad (1),$$

where I is the drug-induced current amplitude, I_{max} is the maximum current amplitude and C is the corresponding drug

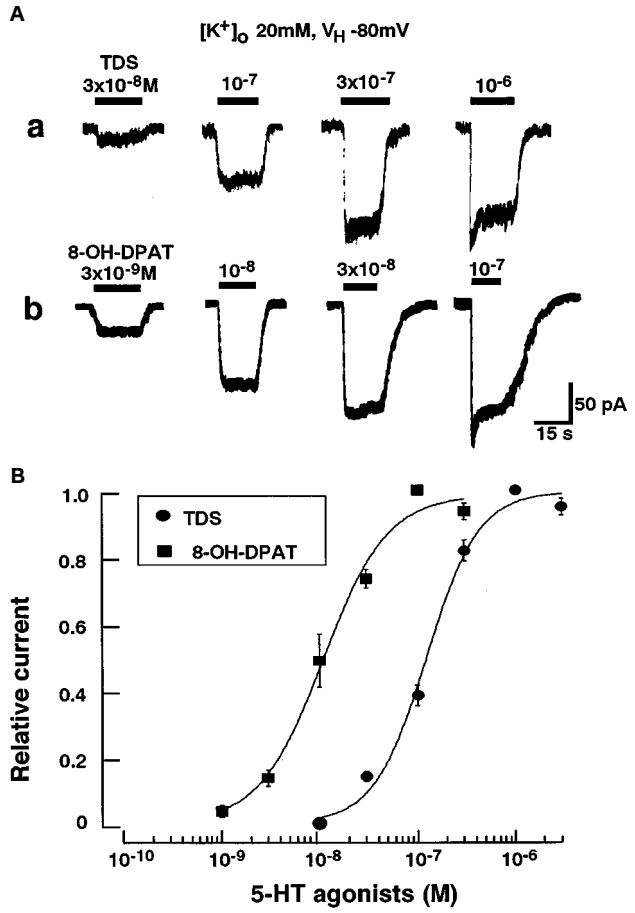

Figure 2 Inward currents induced by TDS and 8-OH-DPAT. All recordings were performed in an external solution containing 20 mM K^+ at a V_H of −80 mV. (A) TDS- (a) and 8-OH-DPAT (b)-induced currents at selected concentrations. (B) The concentration-response relationships for TDS and 8-OH-DPAT. The TDS and 8-OH-DPAT responses were normalized to the peak current induced by 10^{-6} M TDS and 10^{-7} M 8-OH-DPAT, respectively. Each point and the vertical lines represent the mean and s.e.mean from 5 neurones.

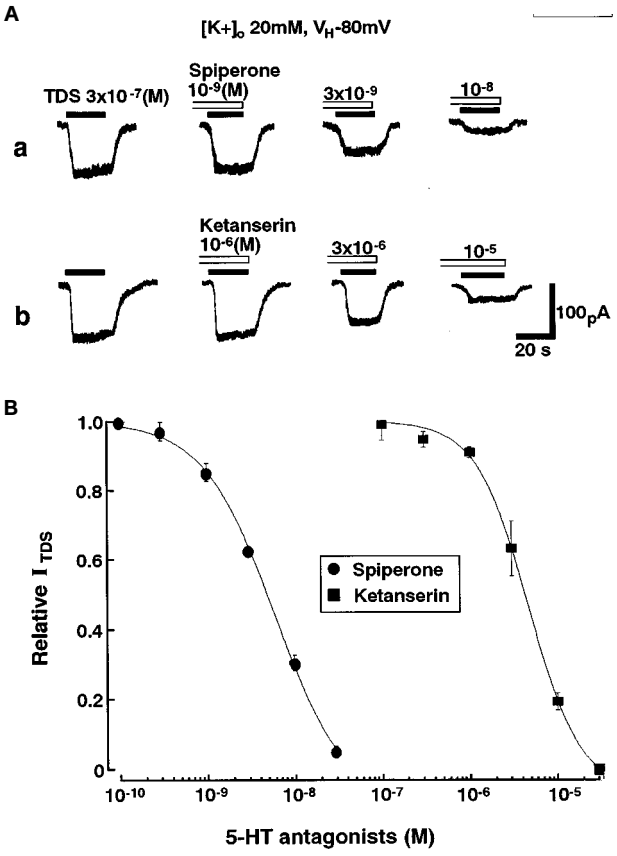

Figure 3 Effects of 5-HT antagonists on TDS-induced currents. All recordings were performed in an external solution containing 20 mM K^+ at a V_H of −80 mV. The neurones were pretreated for 30 s with each 5-HT antagonist before the simultaneous application of 3×10^{-7} M TDS. (A) Inhibition of I_{TDS} by spiperone (a) and ketanserin (b). (B) The concentration-inhibition relationships for the 5-HT antagonists on I_{TDS}. All responses were normalized to the peak current induced by 3×10^{-7} M TDS alone. Each point and the vertical lines represent the mean and s.e.mean from 5 neurones.

concentration. EC_{50} and n_H denote the half-maximum effective concentration and the Hill coefficient, respectively. The data for the concentration-inhibition curve were fitted to the following equation using a least-square fitting routing 2:

$$I = 1 - [C^{n_H}/(C^{n_H} + IC_{50}{}^{n_H}) \quad (2),$$

where I is the current amplitude normalized to the control response without antagonists, C is the concentration of the antagonist, and IC_{50} is the concentration for the half-maximum inhibition.

Results

Activation of inwardly rectifying K^+ currents by TDS

To characterize the TDS responses, whole-cell current recordings were made on the freshly dissociated rat DR neurones using the nystatin perforated patch recording configuration. Under current-clamp conditions, DR neurones showed spontaneous action potentials at frequencies between 0.2–2.5 Hz (1.82±0.22 Hz, $n=7$). The application of TDS hyperpolarized the cell membrane and reduced the spontaneous firing rate of the action potential. The effect of TDS fully recovered after washing out TDS (Figure 1A).

In order to elucidate the ionic mechanism underlying the hyperpolarizing effect of TDS, voltage-clamp experiments were carried out. Figure 1C shows the 3×10^{-7} M TDS-induced currents (I_{TDS}) in normal external solution containing 5 mM-K^+ at various holding potentials (V_Hs). The reversal potential of the I_{TDS} (E_{TDS}) estimated from the I-V relationship was −80.1 mV in the normal external solution with 5 mM K^+. The average value of −80.0±0.23 mV ($n=5$) was almost identical to the theoretical K^+ equilibrium potential (E_k) was −80.3 mV calculated from the known extra- and intracellular K^+ concentrations ($[K^+]_o$ and $[K^+]_i$, respectively) based on the Nernst equation. Figure 1D shows the I-V relationships of the 3×10^{-7} M TDS-induced currents in the external solution with 5, 20 or 50 mM-K^+. The E_{TDS} values for $[K^+]_o$ of 20 and 50 mM were −42.0±0.62 mV ($n=5$) and −24.1±0.52 mV ($n=5$), respectively. The E_{TDS} values shifted 56 mV for a ten fold change in $[K^+]_o$, indicating that the I_{TSD} channels are responsible for selective for K^+. The I-V relationships of I_{TDS} also exhibited inward rectification (Figure 1C, D). The inward rectification becomes evident in the external solution containing K^+ ions of high concentration. Therefore, to record an I_{TDS} having a relatively high signal/noise ratio, all subsequent

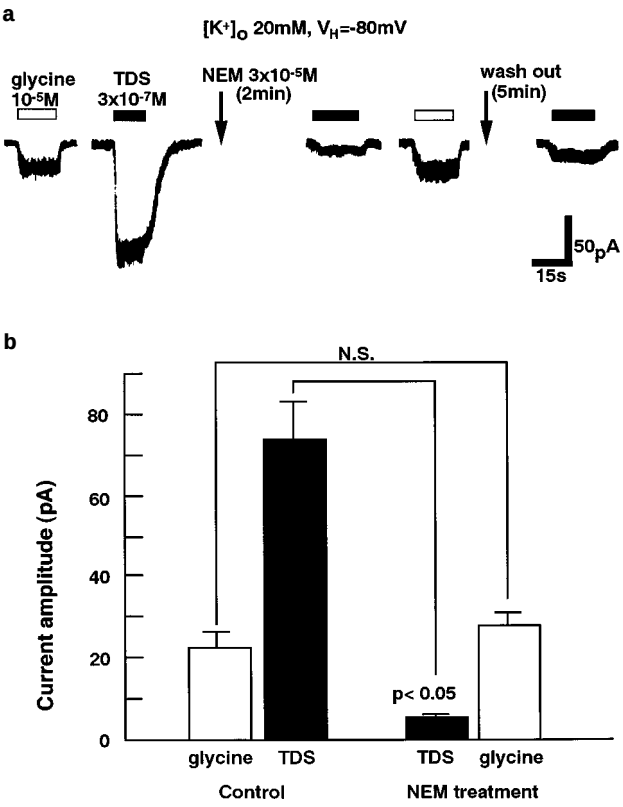

Figure 4 Effects of N-ethylmaleimide. All recordings were performed in external solution containing 20 mM-K^+ at a V_H of −80 mV. (a) The I_{TDS} induced by 3×10^{-7} M TDS was suppressed after the pretreatment for 2 min with 3×10^{-5} M NEM, whereas the 10^{-5} M glycine-induced current was not affected. (b) The amplitude of the TDS- and glycine-induced currents before and after 2 min of treatment with NEM were compared. Each column and the vertical lines represent the mean±s.e.mean from 6 neurones. NS, no significant difference.

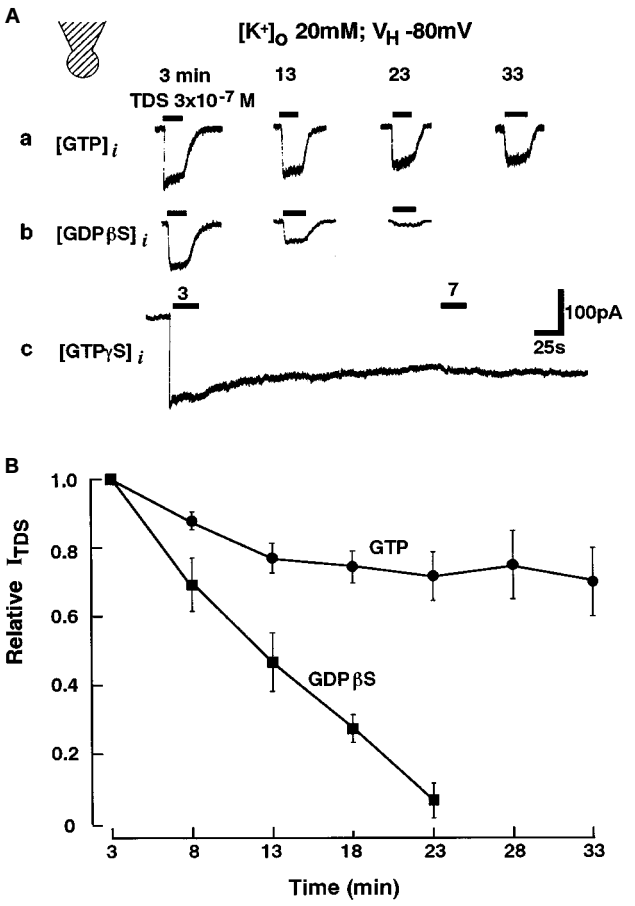

Figure 5 Effects of GDPβS and GTPγS. The neurones were perfused intracellularly with an internal solution containing each nucleotide analogues using the conventional whole-cell patch recording mode. All recordings were performed in an external solution containing 20 mM K^+ at a V_H of −80 mV. All times were measured from the start of the intracellular perfusion by the membrane rupture. (A) Representative recordings of the I_{TDS} after intracellular perfusion with the internal solutions containing 0.3 mM GTP (a), 0.3 mM GDPβS (b) or 0.3 mM GTPγS (c). The horizontal bars indicate the period of 3×10^{-7} M TDS application. The number above the horizontal bar shows the time of intracellular perfusion. (B) The relationship between the time of intracellular perfusion with GTP or GDPβS and the relative peak amplitude of I_{TDS}. All responses were normalized to the peak amplitude of the I_{TDS} with intracellular perfusion for 3 min. Each point and the vertical lines represent the mean and s.e.mean from 5 neurones.

whole-cell current recordings were made in an external solution containing 20 mM K^+.

Effects of 5-HT receptor agonists and antagonists

To identify the subtypes of 5-HT receptor which participate in the induction of I_{TDS}, the effects of 5-HT receptor agonists and antagonists were examined. Figure 2Aa shows the currents induced by TDS at various concentrations in an external solution containing 20 mM K^+ at a V_H of -80 mV. The I_{TDS} increased in a concentration-dependent manner, and a 5-HT_{1A} selective agonist, 8-OH-DPAT, mimicked the TDS effect (Figure 2Ab). The peak amplitudes of these responses are plotted in Figure 2B, in which the EC_{50} and the Hill coefficients were 1.24×10^{-7} M and 0.99 for TDS ($n=5$), and 1.11×10^{-8} M and 0.98 for 8-OH-DPAT ($n=5$), respectively.

The DR neurones were perfused with an external solution containing each 5-HT antagonist for 30 s before the simultaneous application of 3×10^{-7} M TDS with one of the antagonists. A 5-HT_{1A} receptor antagonist, spiperone, reversibly inhibited the I_{TDS} in a concentration-dependent manner with the IC_{50} value of 5.9×10^{-9} M (Figure 3Aa and B). A 5-HT_2 receptor antagonist, ketanserin, also suppressed the I_{TDS} in a concentration-dependent fashion (Figure 3Ab), although the IC_{50} value for ketanserin was 4.6×10^{-6} M (Figure 3B).

Table 1 TDS-induced single channel currents

	GTP (n=6)	*GDP* (n=6)
Mean open time (ms)	0.648±0.05	0.645±0.039
Mean close time (ms)*	104.741±20.674	343.674±54.797
Open probability*	0.008±0.001	0.0017±0.0003
Mean amplitude	1.724±0.079	1.681±0.063

All the data represent mean±s.e.mean of 5 experiments. *Significant difference *versus* the TDS-induced single channel depends on the existence of GTP or GDP in bath solution (Student's paired t test, $P<0.05$).

Effect of N-ethylmaleimide

There is convincing evidence that 5-HT activates 5-HT_{1A} receptors coupled to a G-protein linked directly to the K^+ channel, which hyperpolarizes DR neurones (Penington *et al.*, 1993; Katayama *et al.*, 1997). NEM, a sulphydryl alkylating agent, uncouples the G-protein coupled receptors from the pertussis toxin (PTX)-sensitive G-proteins (G_i and/or G_o) in many tissues including the bovine cerebral cortex, rat sympathetic ganglion cells and the bullfrog atrium (Asano & Ogasawara, 1986; Nakajima *et al.*, 1990; Shapiro *et al.*, 1994). Although the specificity of NEM in targeting G_i and/or G_o is still unclear, NEM at concentrations of 5×10^{-6} to 5×10^{-5} M could uncouple the receptors from G_i and/or G_o without either disturbing agonist-receptor binding (Kilpatrick *et al.*, 1982; Kitamura & Nomura, 1987; Ueda *et al.*, 1990) or blocking the PTX-insensitive signalling pathway (Shapiro *et al.*, 1994). Therefore, we examined whether the I_{TDS} is affected by 3×10^{-5} M NEM. After the perfusion of the external solution containing 3×10^{-5} M NEM for 2 min, the I_{TDS} was significantly reduced as compared with the control (Figure 4A). The blocking action of NEM on I_{TDS} persisted for more than 20 min after washing out the NEM. On the other hand, the inward current induced by 10^{-5} M glycine, was not affected by NEM pretreatment (Figure 4B).

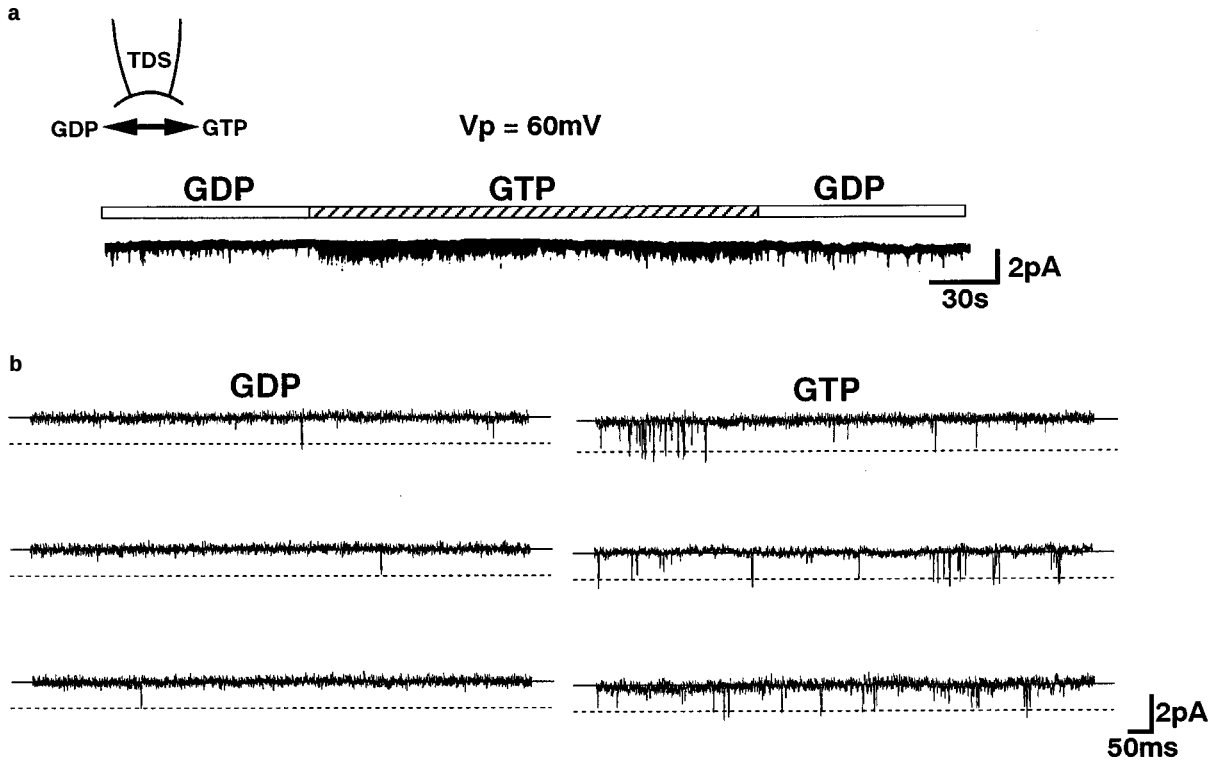

Figure 6 Effect of GDP-GTP exchange on the TDS-induced single-channel activities. The inside-out mode was used to record the TDS-induced single-channel currents. The patch-pipette contained 10^{-7} M TDS. The membrane potential (V_m) was held at -60 mV. (a) GDP or GTP, 0.1 mM, was added to the bathing solution as indicated by horizontal bar above the records. (b) The digitized current traces obtained from (a). The horizontal thin lines show the closed level and the broken lines show the open level.

Effects of nonhydrolyzable guanine nucleotide analogues

In order to make sure of the involvement of G-protein in I_{TDS}, the effects of intracellular perfusion with non-hydrolyzable guanine nucleotide analogues (GDPβS and GTPγS) on the I_{TDS} were investigated using the conventional whole-cell patch recording mode. After the rupture of the patch membrane, we waited 3 min for intracellular perfusion with the pipette solution and then applied 3×10^{-7} M TDS every 5 min. When the neurones were intracellularly perfused with an internal solution containing 0.3 mM GTP (control), the I_{TDS} showed a gradual rundown but maintained 70.1% of the initial current amplitude even at 33 min after the rupture (Figure 5Aa and B). In the neurones perfused intracellularly with 0.3 mM GDPβS, the I_{TDS} decreased rapidly and almost disappeared at 23 min after the rupture (Figure 5Ab and B). The first brief application of TDS resulted in an irreversible activation of K^+ current in the neurones perfused with 0.3 mM GTPγS. The second application of TDS at 7 min after the rupture did not induce any further inward current (Figure 5Ac).

Characterization of TDS-induced single-channel current

The data in Figures 4 and 5 suggest that activation of G-proteins could be involved in the modulation of I_{TDS}. In order to evaluate more directly the relationship between membrane-bound G-proteins and the 5-HT mediated K^+ currents, the single-channel recordings using the inside-out patch recording mode were performed. In patch pipette filled with the external solution without TDS, no single-channel activities were observed under the present experimental conditions. When the patch pipette was filled with the solution containing 10^{-7} M TDS and the cytoplasmic side of the inside-out patch was superfused with the bathing solution containing 0.1 mM GDP, only infrequent openings of single channels were observed at a membrane potential of -60 mV. Perfusion of the cytoplasmic side with 0.1 mM GTP instead of GDP enhanced markedly the TDS-gated single-channel activities (Figure 6 and Table 1), thus indicating the involvement of G-proteins in the TDS response. Figure 7(a and b) shows the TDS-gated single-channel currents (I_{TDS}) at various membrane potentials, in which the cytoplasmic side was bathed with the internal solution containing 0.1 mM GTP. Amplitude histograms were constructed from data obtained at each membrane potential. The mean amplitudes from 5 neurones were used to plot the *I*-V relationship (Figure 7b). The *I*-V relationship represented an inwardly rectifying property of the single-channel currents. The single-channel conductance in the inward direction was 27.7 ± 0.5 pS ($n = 6$).

Discussion

In the present study, we investigated the effects of tandospirone (TDS) on acutely dissociated rat DR neurones. The DR neurones have been classified into two distinct groups based on their firing frequencies and sizes: one has high firing rates (100–130 Hz) and a small soma size and the other has slow spontaneous firing activities (0.5–1.5 Hz) and a large soma size (Park, 1987). The large neurones which exhibited slow firing rates were identified as 5-hydroxytryptaminergic neurones by a histochemical study (Aghajanian & Haigler, 1974). In the present study, the dissociated large DR neurones exhibited a slow rate of spontaneous firing under current-clamp conditions (Figure 1A). The extracellular application of TDS produced a hyperpolarization of the membrane potential

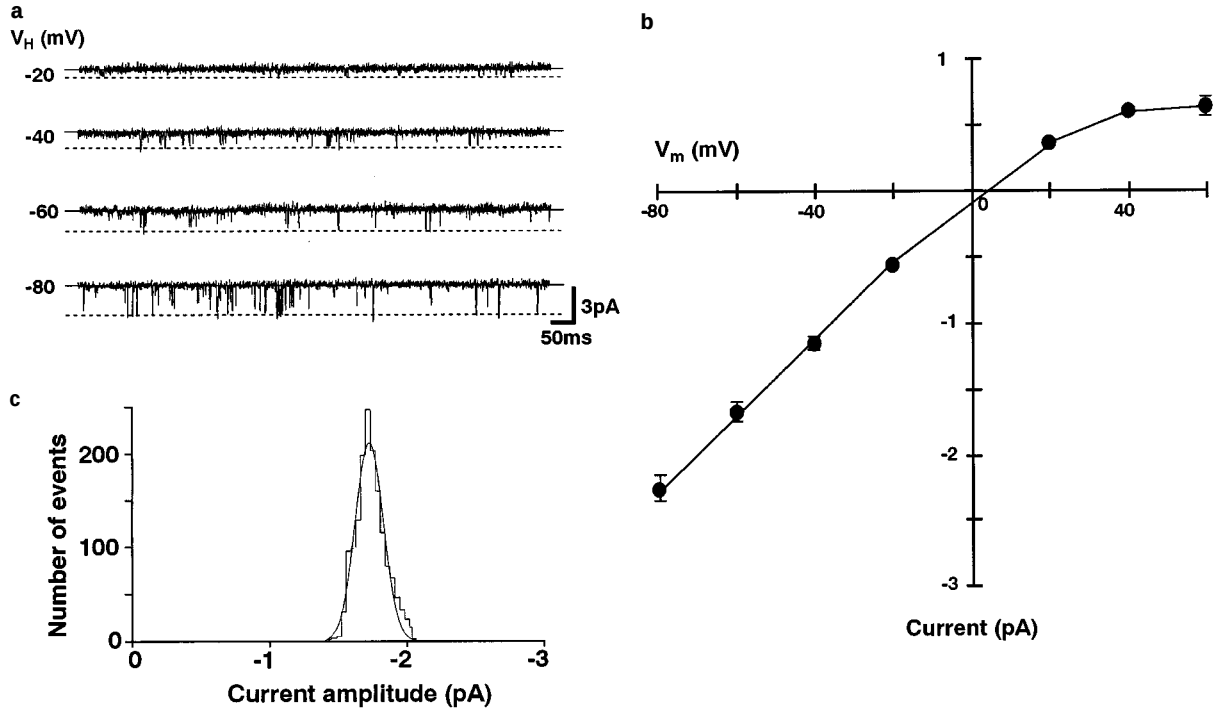

Figure 7 Single-channel currents induced by TDS. (a) The TDS-induced single-channel currents obtained at various membrane potentials. The patch-pipette contained 10^{-7} M TDS. The horizontal thin lines show the closed level while the broken lines show the open level. (b) The *I*-V relationship of the TDS-induced single-channel currents. Each point and the vertical lines show the mean and s.e.mean from 5 neurones. (c) The current amplitude histogram recorded at -60 mV. The number of channel openings were fitted to a Gaussian curve with a peak value at -1.68 pA.

and reduced the firing frequencies (Figure 1A and B). The result is consistent with previous findings that TDS reduced the firing rate of rat DR neurones (Gobert *et al*., 1995).

5-HT receptor-mediated activation of inwardly rectifying K^+ channels has been shown previously in rat DR neurones (Penington *et al*., 1993 Katayama *et al*., 1997). The 5-HT receptor is classified as 5-HT_{1A} subtype by pharmacological methods, and the activation of these 5-HT receptors involves G-protein in the transduction pathway which may interact with the K^+ channel directly (Penington *et al*., 1993; Katayama *et al*., 1997). In the present study, the I_{TDS} was reversibly inhibited by a 5-HT_{1A} receptor antagonist, spiperone, and was mimicked by a 5-HT_{1A} receptor selective agonist, 8-OH-DPAT (Figures 2 and 3). Moreover, TDS elicited K^+ currents which exhibited inward rectification (Figure 1C and D). Inwardly rectifying K^+ currents were also identified in the inside-out single-channel recording (Figure 7). These TDS-induced channel activities were dependent on intracellular GTP acting via G-proteins but not on the soluble second messengers (Figures 5 and 6). In addition, the application of NEM, an uncoupler of PTX-sensitive G-proteins and the receptors (Winslow *et al*., 1987), attenuated the I_{TDS} (Figure 4). These results are in accordance with the properties of the G-protein-activated inwardly rectifying K^+ (GIRK) channels demonstrated in various preparations (Wickman & Clapham, 1995). Our consideration of previous findings and the present results, TDS-mediated action on DR neurones is assumed to be caused by 5-HT_{1A} receptor mediated GIRK channels.

There is considerable information on the regional distribution, cellular localization and molecular structure of the 5-HT_{1A} receptors in mammalian brain (Zifa & Fillion, 1992). The 5-HT_{1A} receptor is located on both the pre- and postsynaptic membranes. The activation of the 5-HT_{1A} receptor has been shown to inhibit the high voltage-activated (HVA) Ca^{2+} channels through activation of PTX sensitive G-proteins in acutely dissociated rat DR neurones (Penington *et al*., 1991) and acutely dissociated rat ventromedial hypothalamic neurones (Rhee *et al*., 1996). Ca^{2+} plays an important role in neurotransmitter release from presynaptic nerve terminals (Heidelberger 1994). Thus, if presynaptic 5-HT_{1A} receptors are coupled to HVA Ca^{2+} channels, the TDS activation of 5-HT_{1A} receptors would reduce the transmitter release from nerve terminals. However, the present study was performed on the K^+ channels of the neuronal cell body and proximal dendrites. Therefore, the effects of TDS on Ca^{2+} channels and the transmitter release from presynaptic nerve terminals are targets for further studies.

Although 5-HT_{1A} receptor agonists and benzodiazepine derivatives have different pharmacological features, both groups of drugs are commonly used as antianxiety drugs (Barrett & Vanover, 1993; Ashton, 1994). Recently, it has been suggested that there is an interaction between diazepam and the 5-hydroxytryptaminergic system (Lopez-Rubalcava *et al*., 1992). Lima *et al*. (1993) showed that benzodiazepine derivatives decreased the activity of the central 5-hydroxytryptaminergic systems. In addition, the release of 5-HT from the hippocampus has been shown to be increased when increased anxiety occurs during benzodiazepine withdrawal (Andrews & File, 1993). The injection of 5-HT_{1A} agonists into the rat hippocampus, an *in vivo* preparation, dose-dependently inhibited the firing of 5-hydroxytryptaminergic DR neurones (Jolas *et al*., 1995). In the slice preparation of rat DR neurones, 5-HT_{1A} agonists activate K^+ channels and suppress the firing activities of 5-hydroxytryptaminergic neurones (Haj-Dahmane *et al*., 1991). These previous studies suggest that the anxiolytic effects of diazepam and 5-HT_{1A} agonists were based on the inhibition of activities of DR 5-hydroxytryptaminergic neurones. In our present experiments, TDS hyperpolarized membrane potential and reduced firing activity of DR neurones by activating the GIRK channels. Therefore, the hyperpolarizing effect of TDS on the DR 5-hydroxytryptaminergic neurones observed in the present study might be one of the most significant mechanisms underlying the anxiolytic effect of TDS.

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