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Taurine-activated chloride currents in the rat sacral dorsal commissural neurons

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Abstract

The electrophysiological and pharmacological properties of taurine (Tau)-activated Cl^- currents (I_{Tau}) were investigated in the dissociated rat sacral dorsal commissural nucleus (SDCN) neurons using the nystatin perforated patch recording configuration under voltage-clamp conditions. The reversal potential of I_{Tau} was close to the Cl^- equilibrium potential. The I_{Tau} was not affected by a preceding GABA response but cross-desensitized by a preceding glycine (Gly) response. Strychnine (STR), picrotoxin (PIC), bicuculline (BIC) and Zn^{2+} suppressed the I_{Tau} in a concentration-dependent manner. The pharmacology of the I_{Tau} and Gly-induced response (I_{Gly}) was similar, though Zn^{2+} inhibition on I_{Tau} differed from that on I_{Gly} in being much slower in recovery. Serotonin potentiated the I_{Tau} via protein kinase C. The results indicate that both Tau and Gly act on a strychnine-sensitive site to open the same Cl^- channels in the SDCN neurons, and suggest that Tau may act as a functional neurotransmitter in the mammalian SDCN. © 1998 Elsevier Science B.V.

Keywords: Taurine; Sacral dorsal commissural nucleus; Acutely dissociated neurons; Nystatin perforated patch clamp; Chloride current; Strychnine-sensitive glycine receptor

1. Introduction

Taurine (Tau) is an endogenous amino acid involved in many physiological functions such as development and osmoregulation [8,10]. The high concentrations of Tau in the central nervous system suggest a specific role as a neurotransmitter for this amino acid, although this has yet to be firmly established at any central synapse. The biochemical and functional roles of Tau have been intensely studied in the cerebellum and substantia nigra because Tau exists most densely in these brain regions and it has been proposed as a possible inhibitory neurotransmitter [6,11,22,23]. The Tau immunopositive axon terminals were found in the dorsal horn [17]. There is also functional evidence for an inhibitory action of Tau on spinal cord neurons [20,3]. Ionophoretically-applied Tau inhibits neurons in the spinal cord in vivo [4]. Intrathecally-applied Tau selectively inhibits substance P-induced biting and scratching behaviour [29]. Although glycine (Gly) has generally been considered a major component of the nociceptive pathway in the spinal cord, Tau may also be involved in modulating transmission of nociceptive information [7]. It is well known that Tau acts mainly on the Gly receptor–Cl^- channel complex in substantia nigra neurons [5,6,11,22,36], but it is not clear whether this is true for the neurons of the sacral dorsal commissural nucleus (SDCN) [34].

The main purpose of this study is to elucidate the electrophysiological and pharmacological properties of Tau-activated whole-cell Cl^- currents (I_{Tau}) in the acutely dissociated SDCN neurons using the nystatin perforated patch recording configuration and compare its action to that of Gly.

2. Materials and methods

2.1. Preparation

The SDCN neurons were acutely dissociated as described elsewhere [34]. Briefly, 2-week-old SD rats were decapitated under pentobarbitone sodium anesthesia (50 mg kg^{-1}, i.p.). A segment of the lumbosacral (L6-S2)

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spinal cord was dissected out and sectioned with a vibratome tissue slicer (DTK-1000; Dosaka, Kyoto, Japan) to yield several transverse slices (400-μm thick) containing the SDCN region. The slices were preincubated in oxygenated incubation solution (see below) for 50 min at room temperature (22–25°C). Thereafter, slices were treated enzymatically in oxygenated incubation solution containing 1 mg/6–8 ml pronase for 20 min at 31°C followed by exposure to 1 mg/6–8 ml thermolysin for another 15 min in the same conditions. After the enzyme treatment, the slices were kept in enzyme-free incubation solution for 1 h. Then a portion of SDCN region was micro-punched out with an electrolytically polished injection needle and transferred into a culture dish filled with standard external solution (see below). Neurons were mechanically dissociated with fire-polished Pasteur pipettes under visual guidance under a phase contrast microscope (Olympus, IX70). Dissociated neurons adhered to the bottom of the dish within 20 min, allowing the electrophysiological studies to be conducted. The neurons that retained their original morphological features, such as the dendritic processes, were then used for the experiments.

2.2. Solutions

The composition of incubation solution was (mM): NaCl, 124; $NaHCO_3$, 24; KCl, 5; KH_2PO_4, 1.2; $CaCl_2$, 2.4; $MgSO_4$, 1.3; glucose, 10; aerated with 95% O_2–5% CO_2 to a final pH of 7.4. The normal external standard solution contained (mM): NaCl, 150; KCl, 5; $CaCl_2$, 2; $MgCl_2$, 1; *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulphonic acid (HEPES), 10; glucose, 10; pH was adjusted to 7.4 with tris-(hydroxymethyl)-aminomethane (Tris-base). The patch pipette solution for nystatin perforated patch recording was (mM): CsCl, 150; HEPES, 10; pH was adjusted to 7.2 with Tris-base. A nystatin stock solution dissolved in acidified methanol at a concentration of 10 mg/ml was prepared and stored at −20°C. The stock solution was added to the patch-pipette solution just before use to give a final nystatin concentration of 400 μg/ml. When the current–voltage (I–V) relationship for I_{Tau} was examined, 0.3 μM tetrodotoxin (TTX) and 10 μM $CdCl_2$ were added to the standard external solution. $CdCl_2$ had no effect on the I_{Tau} at the concentration used.

2.3. Perforated patch recording

Electrical measurements were carried out by using nystatin perforated patch recording configuration under voltage-clamp conditions at room temperature (22–25°C). Patch pipettes were pulled from glass capillaries with an outer diameter of 1.5 mm (Narishige) on a two-stage puller (PB-7; Narishige, Tokyo, Japan). The resistance between the recording electrode filled with pipette solution and the reference electrode was 4–6 MΩ. The patch pipette was positioned on a neuron using a hydraulic micromanipulator (WR-3; Narishige). The electrode was connected to a patch clamp amplifier (CEZ-2300, Nihon Koden, Tokyo, Japan). The current and voltage were monitored with a pen recorder (Omniace RT 3100, San-ei, Japan), filtered at 1 kHz and sampled and analysed using a DigiData 1200A interface and a computer with pCLAMP 6.0.2 program (Axon Instruments, USA). The membrane potential was held at −40 mV throughout the experiment, except when examining the I–V relationships. All measurements were started after stabilization of the Tau responses (15–25 min after cell attachment).

2.4. Drugs and their application

Pronase was purchased from Calbiochem. Chelerythrine and phorbol 12-myristate 13-acetate (PMA) were from Research Biochemicals International. Other drugs were from Sigma. All drugs were dissolved in distilled water and further diluted to their final concentrations in standard external solution just before use. Drugs were applied via a 'Y-tube' [21]. This system allows a complete exchange of external solution surrounding a neuron within 20 ms.

2.5. Statistics

Data were calculated as the mean ± standard error (S.E.M.). The continuous theoretical curves for concentration–response relationships of Tau and Gly were drawn according to a modified Michaelis–Menten Eq. (1) using a least-square fitting routine (Newton–Raphson method) after normalizing the amplitude of the response:

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

where I is the normalized value of the current, I_{max} the maximal response, C the drug concentration, EC_{50} the concentration which induced the half-maximal response, and n the apparent Hill coefficient. The continuous lines for concentration–inhibition curves of strychnine, picrotoxin, bicuculline and Zn^{2+} were drawn according to the following equation:

$$I = IC_{50}^n / (C^n + IC_{50}^n) \quad (2)$$

where I represents the fraction of a current that remains after strychnine, picrotoxin, bicuculline or Zn^{2+} treatment.

3. Results

3.1. Tau-activated currents in SDCN neurons

The SDCN neurons were voltage-clamped at a holding potential (V_H) of −40 mV. The application of Tau and Gly evoked inward currents in all SDCN neurons tested (Fig. 1A). The Tau currents became detectable at a concentration of about 3×10^{-5} M and then increased in a sigmoidal fashion with increasing Tau concentration and the maximal response concentration was 3×10^{-3} M. At concentrations higher than 10^{-4} M, Tau responses showed

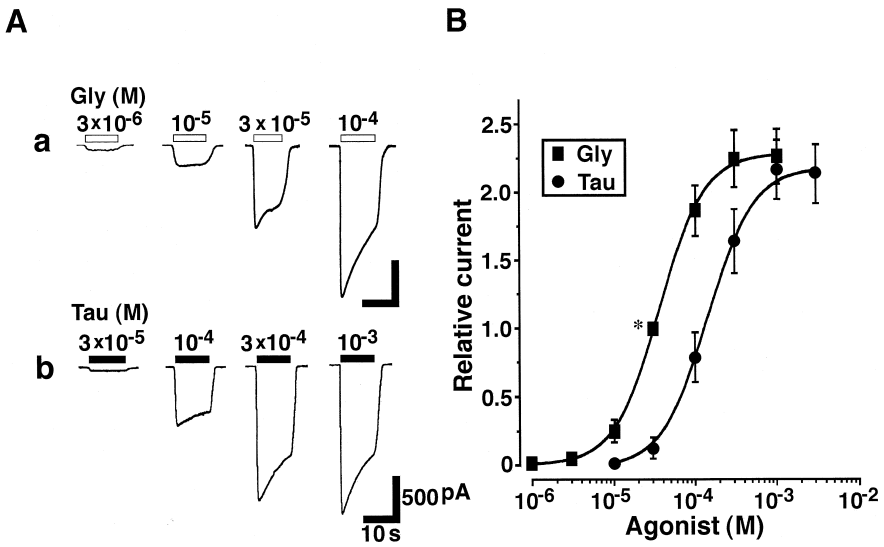

Fig. 1. Gly and Tau responses in dissociated SDCN neurons. (A) Inward currents induced by Gly and Tau at various concentrations. (B) Concentration–response relationships for Gly- and Tau-activated current (I_{Gly} and I_{Tau}). All currents were normalized to the peak current amplitude induced by 3×10^{-5} M Gly (*). Each point represents the mean of six neurons. Here and in subsequent figures the vertical bars show ±S.E.M.

desensitization. The concentration–response relationships of Gly- and Tau-activated currents (I_{Gly} and I_{Tau}) are summarized in Fig. 1B. All responses were normalized to the peak response elicited by 3×10^{-5} M Gly. The EC_{50} value and the Hill coefficient were 1.28×10^{-4} M and 1.33 for I_{Tau}; 3.46×10^{-5} M and 1.35 for I_{Gly}, respectively. The concentration range for I_{Tau} is higher than that of Gly in the SDCN neurons [34].

3.2. I–V relationship for I_{Tau}

In order to determine the ion that is responsible for I_{Tau} in the acutely dissociated SDCN neurons, the I–V relationship for I_{Tau} was examined. The dissociated neurons were perfused with the external and internal solutions containing 161 and 150 mM Cl^- ($[Cl^-]_o$ and $[Cl^-]_i$), respectively. Fig. 2A illustrates the currents induced by 10^{-4} M Tau at various V_Hs. The reversal potential of I_{Tau} (E_{Tau}) was $+1.69 \pm 1.1$ mV ($n = 6$), which was close to the theoretical Cl^- equilibrium potential (E_{Cl}) of -1.8 mV calculated from the given extra- and intracellular Cl^- activities with the Nernst equation (Fig. 2B). The reversal potential of I_{Gly} (E_{Gly}) was $+1.51 \pm 1.2$ mV ($n = 4$), which was also close to the E_{Cl} of -1.8 mV. These results indicate that both I_{Tau} and I_{Gly} are carried by Cl^-.

3.3. Cross-desensitization between Tau and Gly responses

The cross-desensitization between Tau- and either GABA- or Gly-induced currents was examined. Fig. 3A

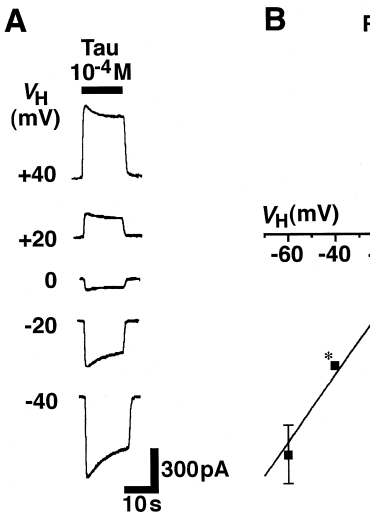

Fig. 2. The current–voltage (I–V) relationship for I_{Tau}. (A) Currents induced by 10^{-4} M Tau at various V_Hs. (B) I–V relationship for I_{Tau}. E_{Cl} represents the Cl^- equilibrium potential. Currents were normalized to the peak I_{Tau} at V_H of -40 mV (*). Each point represents an average from six neurons.

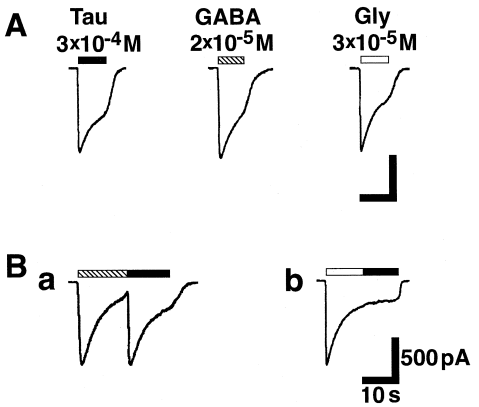

Fig. 3. Cross-desensitization of Tau and Gly responses. (A) Tau (3×10^{-4} M, filled bar), GABA (2×10^{-5} M, dotted bar) and Gly (3×10^{-4} M, open bar) produced inward currents similar in shape and amplitude to each other. (B) I_{Tau} was not affected by the preceding GABA response (a) but desensitized completely by the Gly response (b).

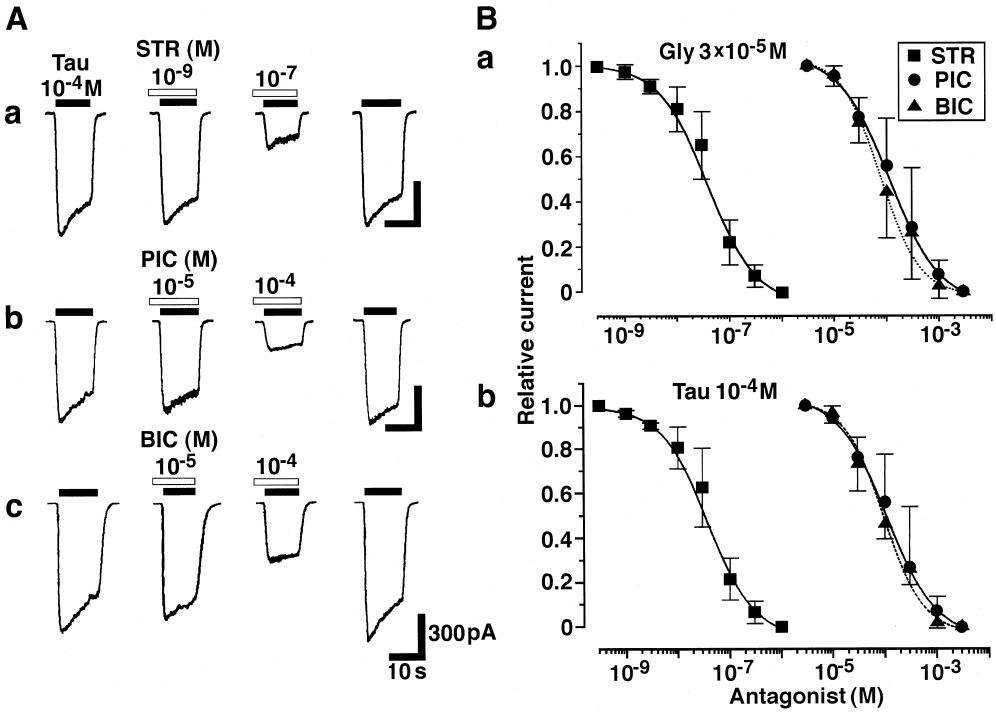

Fig. 4. The inhibition of I_{Tau} by strychnine (STR), picrotoxin (PIC) and bicuculline (BIC). (A) I_{Tau} was inhibited by (a) STR, (b) PIC and (c) BIC in a concentration-dependent manner. Data in (a), (b) and (c) were from different neurons. (B) concentration–inhibition curves for STR, PIC and BIC on 3×10^{-5} M Gly- and 10^{-4} M Tau-induced Cl^- currents. All antagonists were perfused for 30 s before simultaneous application of Gly or Tau. The amplitudes of I_{Gly} and I_{Tau} were measured at the peak and expressed as relative values to the control response induced by 3×10^{-5} M Gly and 10^{-4} M Tau alone (for each antagonist, $n = 5–7$), respectively.

shows that concentrations of Tau (3×10^{-4} M), GABA (2×10^{-5} M), and Gly (3×10^{-5} M) induced very similar currents in the amplitude to each other at a V_H of -40 mV. The response to a subsequent application of Tau after GABA was almost similar in form and amplitude to that of Tau alone (Fig. 3Ba), indicating that there is little cross-desensitization between GABA and Tau responses in the SDCN neurons. On the other hand, the I_{Tau} was com-

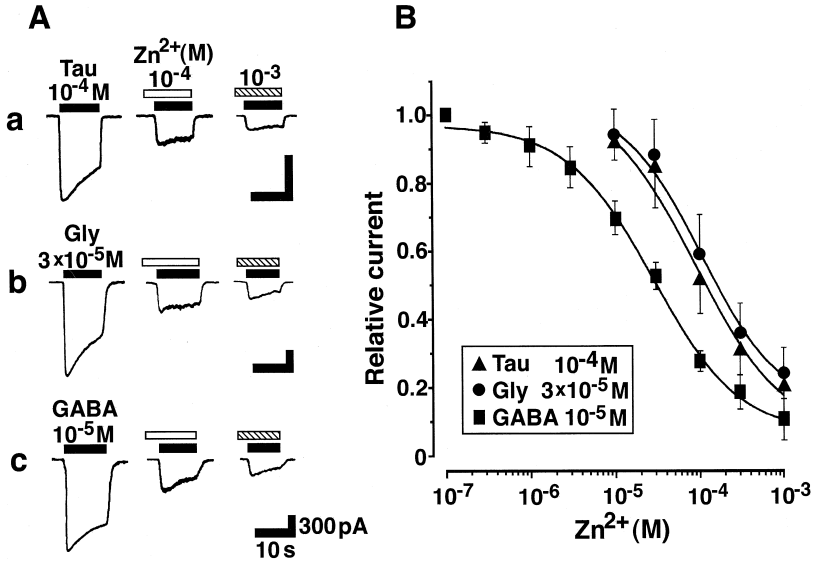

Fig. 5. The effects of Zn^{2+} on GABA-, Gly- and Tau-induced Cl^- currents. (A) Current traces illustrating the effects of 10^{-4} and 10^{-3} M Zn^{2+} on (a) Tau-, (b) Gly- and (c) GABA-induced currents. (B) Zn2 + depression of the whole-cell currents elicited by 10^{-4} M Tau, 3×10^{-5} M Gly and 10^{-5} M GABA. The relative change in amplitude compared with the control response seen in the absence of Zn^{2+} is plotted against extracellular Zn^{2+} concentration. Each point represents the mean of five experiments.

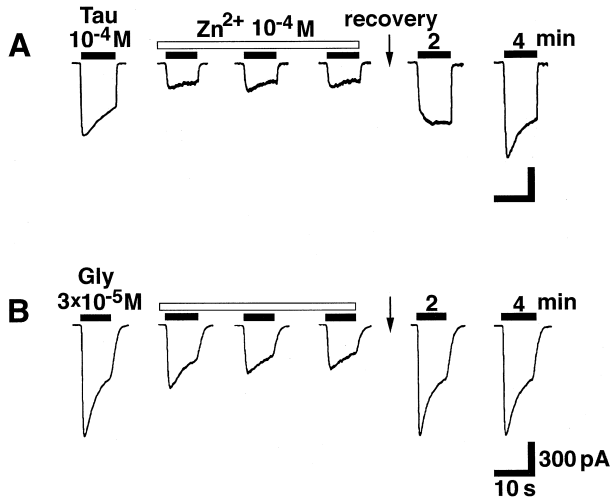

Fig. 6. Recovery of I_{Tau} and I_{Gly} after Zn^{2+} inhibition. (A) and (B) were obtained from the same neuron. Similar results were observed in another four cells.

pletely inhibited by a preceding Gly response (Fig. 3Bb). Such strong cross-desensitization between Gly and Tau was confirmed in all of the SDCN neurons examined ($n = 5$).

3.4. Inhibition of I_{Tau} by strychnine, picrotoxin and bicuculline

In this experiment, a selective Gly receptor antagonist strychnine (STR) inhibited the I_{Tau} in a concentration-dependent manner with an IC_{50} value of 3.73×10^{-8} M (Fig. 4Aa, Bb). On the other hand, the inhibitory effects of picrotoxin (PIC) and bicuculline (BIC) on the I_{Tau} became evident only at the high concentrations above 10^{-5} M. The IC_{50} values obtained from the inhibition curves were 1.07×10^{-4} M for PIC and 9.39×10^{-5} M for BIC, respectively (Fig. 4Ab,Ac,Bb). Fig. 4Ba shows the inhibition curves of STR, PIC and BIC on I_{Gly} and their IC_{50} values are similar to those for inhibiting I_{Tau}.

3.5. Effect of Zn^{2+} on I_{Tau}

At physiological concentrations Zn^{2+} is a non-competitive antagonist of $GABA_A$ receptors [31,18,26,12]. Zn^{2+} also affects the function of the strychnine-sensitive Gly receptors [1,16,37]. In this experiment, the currents activated by Tau, Gly and GABA were all inhibited by extracellularly applied Zn^{2+} in a concentration-dependent manner (Fig. 5A). The IC_{50} values obtained from the inhibition curves were 8.68×10^{-5} M for Tau, 1.04×10^{-4} M for Gly and 2.80×10^{-5} M for GABA, respectively (Fig. 5B). It has been reported that low concentrations of Zn^{2+} potentiated both I_{Gly} and I_{Tau}. In the SDCN neurons, however, neither I_{Gly} nor I_{Tau} was affected by low concentrations of Zn^{2+}. The recovery of Zn^{2+} inhibition on I_{Tau} was relatively slow though complete after 4–6 min of wash (Fig. 6A). This recovery time course is different from the effect of Zn^{2+} on Gly response where the inhibition occurs with a fast recovery (Fig. 6B).

3.6. Potentiation of I_{Tau} by 5-HT

The pharmacology of I_{Tau} suggested that Tau can act on strychnine-sensitive Gly receptors to open the same Cl^- channels in SDCN neurons. Since we previously

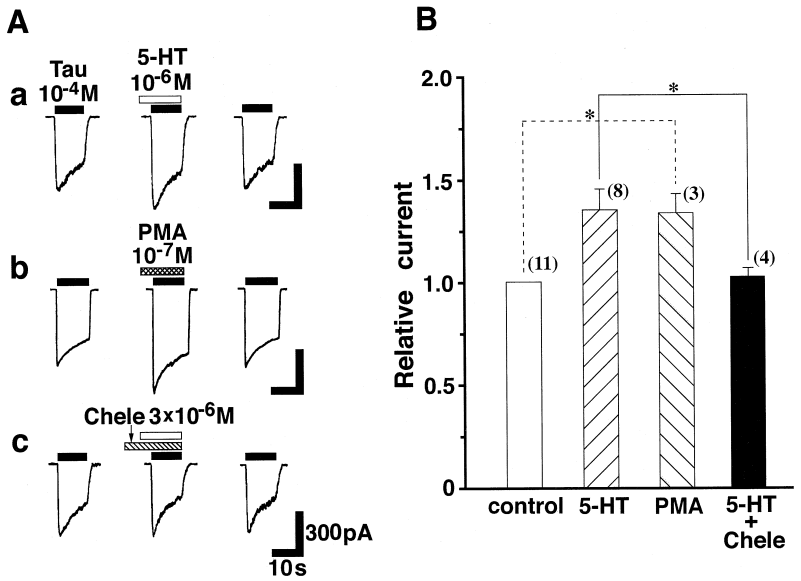

Fig. 7. The potentiation of I_{Tau} by 5-HT. (A) Pretreatment with 10^{-6} M 5-HT for 3–5 min potentiated I_{Tau} induced by 10^{-4} M Tau (a). PMA (10^{-7} M) mimicked the 5-HT effect on I_{Tau} (b). In the presence of 3×10^{-6} M chelerythrine, the facilitatory effect of 5-HT disappeared (c). (B) Numerical data obtained from 3–11 cells. Ordinate indicates the normalized amplitude of I_{Tau}. The control I_{Tau} measured before application of drugs is taken as 1. $* P < 0.01$.

observed that serotonin (5-hydroxytrptamine, 5-HT) augmented the I_{Gly} in the SDCN neurons through protein kinase C (PKC) [34], the effect of 5-HT on I_{Tau} was examined. In the present experimental conditions for recording the I_{Tau} in the SDCN neurons, pretreatment with 5-HT alone induced no noticeable current at concentrations up to 10^{-4} M. However, the application of 10^{-6} M 5-HT gradually enhanced the peak amplitude of 10^{-4} M Tau-induced currents. The maximal potentiation appeared 3–5 min after the start of the 5-HT application ($135.1 \pm 10.3\%$ compared with that before 5-HT application, $n = 8$, $P < 0.01$, Fig. 7Aa,B). The effect of 5-HT was reversible with washing out the 5-HT in the bathing medium. PMA (10^{-7} M), a PKC activator, mimicked the 5-HT effect on I_{Tau} (Fig. 7Ab,B). After the neurons were loaded with 3×10^{-6} M chelerythrine (Chele), a novel PKC inhibitor, the application of 10^{-6} M 5-HT failed to enhance I_{Tau} (Fig. 7Ac,B).

4. Discussion

This study demonstrates that Tau acts upon strychnine-sensitive Gly receptors and increases the membrane Cl^- conductance in the acutely dissociated SDCN neurons. Present cross-desensitization experiments suggest that Tau and Gly act upon the same recognition site in these cells. These findings extend those from in vivo studies of the inhibitory effect of Tau on the spinal cord neurons, suggesting that Tau as well as Gly may act as a functional neurotransmitter in the mammalian SDCN.

In these experimental conditions, the conclusion that both Tau and Gly act on a strychnine-sensitive site to open the same Cl^- channels in SDCN neurons is based on the following evidence. Firstly, the reversal potential of I_{Tau} was comparable to that of I_{Gly} in these neurons, and the I_{Tau} was antagonized completely by the Cl^- channel blocker, picrotoxin. Secondly, there is a strong cross-desensitization between Gly and Tau responses while little cross-desensitization exists between GABA and Tau responses. Thirdly, the effects of Tau and Gly were pharmacologically indistinguishable with both being sensitive to strychnine, yet neither were antagonized by bicuculline at concentrations that block $GABA_A$ receptor-mediated response on these cells. Activation of the same conductance by Gly and Tau has been previously shown in the isolated hypothalamic [30], substantia nigra [11,22,23], medullary and hippocampal neurons [13]. In addition, single-channel studies in cultured spinal cord neurons [20] showed that Tau activates Cl^--dependent channels with similar properties to those activated by Gly. It may be that both amino acids act upon identical recognition sites on the same set of Gly receptor–Cl^- channel marcromolecules.

Zn^{2+} is an endogenous cation which exists throughout the mammalian central nervous system (CNS) [28]. Zn^{2+} is stored in synaptic vesicles and can be released from presynaptic terminals in large quantities during synaptic activity [2]. Both $GABA_A$ and NMDA receptors on CNS neurons can be inhibited by Zn^{2+} [31], and Zn^{2+} can modulate synaptic transmission [32]. Inhibition of $GABA_A$ receptors by Zn^{2+} is non-competitive, leading to the proposal of a distinct extracellular Zn^{2+} binding site [26,12]. It has been reported recently that Zn^{2+} at concentrations from 100 nM to 10 μM enhances Gly-induced current about 3-fold in cultured spinal cord neurons [16]. A similar modulation of Gly response by Zn^{2+} was also found with recombinant homo- and hetero-oligomeric Gly receptors generated in *Xenopus* oocytes [1,16]. In contrast, Gly-induced response in ciliary ganglion neurons showed only a slight potentiation by Zn^{2+} at low concentrations [37]. In the SDCN neurons, we showed neither I_{Gly} nor I_{Tau} was affected by low concentrations of Zn^{2+}. This apparent discrepancy may result from the heterogeneity of the subunit composition of Gly receptors [1,16]. Nevertheless, Zn^{2+} at high concentration ($> 10^{-5}$ M) inhibited both I_{Gly} and I_{Tau} in SDCN neurons. The IC_{50} values for Gly and Tau responses were much the same but they differed from that for the GABA response. Thus different inhibitory mechanisms may be involved. Interestingly, the Zn^{2+} inhibition on I_{Tau} differed from that on I_{Gly} in being much slower to recovery. The magnitude of the inhibitory action of Zn^{2+} was not changed by repeated application of Tau in the continued presence of Zn^{2+} (Fig. 6A), indicating that the Zn^{2+} blockage is not use-dependent. There are reports of differential sensitivities of the Tau and Gly conductances to various antagonists, including strychnine [9], which may reflect the presence of different receptor isoforms in different areas of the brain [14]. Indeed, it has been reported that the sensitivity of cloned Gly receptors expressed in *Xenopus* oocytes to both Gly and strychnine can be profoundly reduced by exchange of a single amino acid on the $\alpha 2$ subunit, while sensitivity to Tau appears to be unaffected [15]. In view of its effects on I_{Tau} in SDCN neurons, Zn^{2+} may have modulatory consequences for synaptic transmission not previously recognized.

The I_{Tau} in the SDCN neurons was potentiated by serotonin via intracellular PKC. Whether Tau and serotonin act as cotransmitter in the SDCN remains to be further elucidated. The SDCN neurons exhibit the receptors for excitatory amino acids [33,35], substance P [19], serotonin [34], norepinephrine [24] and Gly [34] as well as Tau (this study). The NMDA and AMPA/KA receptor-channel complexes as well as Gly receptors are thus regulated in their activities by various intracellular protein kinases [25,27]. In addition, the membrane receptors that couple to G proteins modulate the production and activation of intracellular protein kinases. For example, substance P and serotonin receptors as well as adrenoceptor activation all result in the regulation of intracellular PKC and protein kinase A (PKA). In this sense, the efficacy of excitatory and inhibitory afferents on the SDCN neurons is

regulated not only by presynaptic factors, but by the intracellular modulation of postsynaptic receptors.

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