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Effects of the P_2-purinoceptor antagonists suramin and pyridoxal-phosphate-6-azophenyl-2′,4′-disulfonic acid on glutamatergic synaptic transmission in rat dorsal horn neurons of the spinal cord

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Abstract

The effects of suramin and pyridoxal-phosphate-6-azophenyl-2′,4′-disulfonic acid (PPADS) on glutamatergic synaptic transmission were studied on dorsal horn lamina II neurons of rat spinal cord slice preparation and cultured dorsal horn neurons. Suramin at 100 μM significantly suppressed the amplitude of the evoked excitatory postsynaptic currents (EPSCs) by 33%, miniature EPSC (mEPSC) amplitude was decreased by 46% and the mEPSC frequency also decreased by 41%. PPADS at 50 μM had little effect on either the evoked EPSCs or mEPSCs. The lack of effect of PPADS on glutamatergic synaptic transmission suggests that the effect of suramin is less likely to be mediated by P_{2x} receptors. When whole-cell (±)-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) currents evoked by glutamate were examined, both suramin and PPADS showed no inhibition of peak amplitude. However, the onset of glutamate-evoked whole-cell currents became significantly slowed by suramin but not by PPADS. The suppression of synaptic transmission by suramin may be due, in part, to the slowed onset of glutamate-evoked AMPA currents. These results suggest that the analgesic effects of suramin shown in cancer patients and animal pain models may not be solely due to its antagonism to purinoceptors. PPADS is probably a more suitable antagonist for the study of synaptic $P2_x$ receptor function at excitatory synapses mediated by AMPA receptors.

Keywords: ATP receptors; P_{2x} receptors; Synaptic transmission; Pyridoxal-phosphate-6-azophenyl-2′,4′-disulfonic acid; Suramin; Glutamate; (±)-α-Amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors; Spinal cord

P_{2x}-purinoceptors (P_{2x} receptors) are a large family of ligand-gated cation channels for which ATP is the endogenous ligand [4,19]. They have been found to be expressed in peripheral tissues [4], sensory neurons [5,16] and the central nervous systems (CNS) [2–4,6,20]. P_{2x} receptors may be involved in nociceptive sensory transmission [5,7]. Studies have shown that P_{2x} receptor antagonist, suramin, could ameliorate painful sensation in cancer patients and animals [10,14]. Since P_{2x} receptors are expressed on presynaptic terminals of dorsal root ganglion (DRG) neurons and their activation enhances glutamate release [13], the analgesic effect of suramin may be mediated by antagonizing these receptors. In addition, P_{2x} receptors expressed on peripheral terminals of nociceptive DRG neurons [7] may be targets for suramin as well. However, here we found that suramin's effects were complicated by its non-specific actions on glutamatergic synaptic transmission.

Transverse cervical spinal cord sections (350-400 mm) from postnatal (P6-P14) rats were acutely prepared as previously described [1]. Whole-cell patch-clamp recordings were obtained from dorsal horn lamina II neurons voltage-clamped at −70 mV. Excitatory postsynaptic currents (EPSCs) were evoked by focal stimulation (10–50 V, con-

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stant-voltage) of the nearby tissue at 0.033–0.1 Hz for 0.1-0.3 ms in the presence of 10 μM bicuculline and 5 μM strychnine [1]. The evoked EPSCs included in this study were mediated by AMPA receptors as evidenced by their rapid kinetics and complete block by 10 μM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) (tested in 6/8 cells; bottom trace in Fig. 1). Fig. 1 shows an example of the effect of 100 μM suramin on the evoked EPSCs recorded from a neuron of a P9 rat. The amplitudes of the evoked EPSCs were decreased by suramin at 100 μM and 200 μM (Fig. 1 top traces). In four cells tested with 100 μM suramin, there was a significant decrease (33.4 ± 12.1%, $n = 4$) of the evoked EPSC amplitude. The decrease of evoked EPSCs by suramin was reversible following a wash with a normal bath, as is shown in Fig. 1. This inhibitory effect appeared to be consistent with an inhibition of presynaptic P_{2x} receptors. However, when PPADS (50 μM) was tested on the same cells as those tested with suramin following recovery, no decrease in the amplitude of the evoked EPSC was observed (Fig. 1 bottom traces; $n = 4$). The discrepancy between the effects of these two P_{2x} re-ceptor antagonists suggested two possibilities. First, the presynaptic P_{2x} receptors may be insensitive to PPADS. However, this possibility is discounted because our previous study showed that presynaptic P_{2x} receptors could be completely blocked by 50 mM PPADS [13]. The second possibility is that the effect of suramin was due to a non-specific effect on glutamatergic synaptic transmission. Support for this possibility is given by the observation that suramin inhibits whole-cell currents evoked by GABA, glycine, NMDA, and kainate [8,18].

To test whether suramin inhibits AMPA receptor function directly, we determined the effect of suramin on glutamate-evoked whole-cell currents in a bath with 2 mM Mg^{2+} and no added glycine. Under these conditions, glutamate-evoked currents are predominantly due to activation of AMPA receptors [11,13]. Effects of suramin and PPADS on glutamate-evoked whole-cell currents were analyzed by

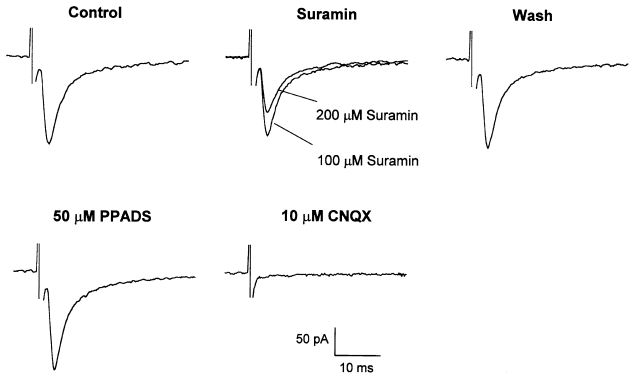

Fig. 1. Effects of suramin and PPADS on the amplitude of evoked EPSCs recorded from lamina II neurons of spinal cord slices The top three traces show the evoked EPSCs before (control), in the presence of 100 μM and 200 μM suramin, and after wash off of suramin. On the same cell, the bottom two traces show evoked EPSCs in the presence of 50 μM PPADS and after including 10 μM CNQX in bath solution. Each trace represents an average of 15 evoked EPSCs.

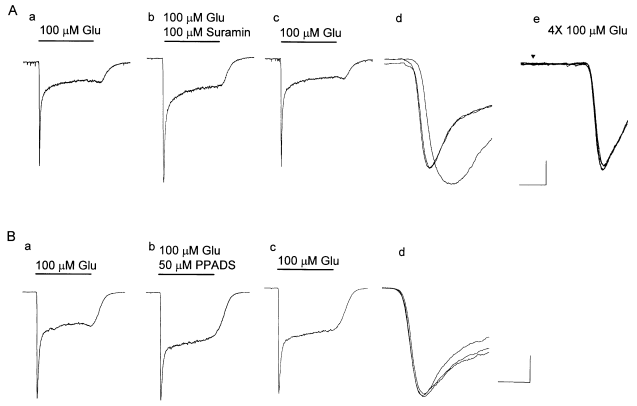

Fig. 2. Effects of suramin and PPADS on glutamate-evoked whole-cell currents (A). 100 μM glutamate-evoked whole cell currents in normal bath solution (Aa), after the cell was treated with 100 μM suramin for 2 min (Ab), and following wash (Ac). A small portion of the three traces (Aa, Ab and Ac) is at an expended time scale (Ad). In another cell (Ae) whole-cell currents were evoked at four different times with 100 μM glutamate. The traces (Ad and Ae) are aligned at the time when the solenoid valve was opened (triangle in Ae) for delivering glutamate onto the cells. Vertical scale bar: 200 pA for Aa–Ad, 300 pA for Ae. Horizontal bar: 1 s for Aa–Ac, 30 ms for Ad, and 42.5 ms for Ae. (B) Whole-cell currents evoked by 100 μM glutamate in normal bath (Ba), following treatment with 50 μM PPADS for 2 min (Bb), and after 30 min wash with normal bath (Bc). Bd shows an expanded view of the early part of the three traces in Ba–Bc, which are aligned at the time when the solenoid valve was opened for delivering glutamate onto the cell. Vertical scale bar: 200 pA for Ba–Bd. Horizontal bar: 1 s for Ba–Bc, 40 ms for Bd. Drugs were applied with a gravity-driven fast flow device which delivered drugs onto a recording neuron through glass tubes (inner diameter, 1.1 mm). The lowpass filter of the recording amplifier was set at 2 kHz and the traces were sampled at 4 kHz.

determining changes of the peak and steady-state currents, the onset of glutamate action, and the time constant of current decay from peak to steady state. The amplitude of steady state currents was measured at the end of a 2-s application of glutamate. The onset of glutamate action was measured as the time from triggering of drug delivery to the time when digitized whole-cell current values became greater than baseline noise on the rising phase of the glutamate current response. Rise times were measured between 10 and 90% of peak currents. The time constant of current decay from peak to steady state was determined by fitting a single exponential curve to the decay phase (10–90% of peak currents above steady state currents) of glutamate-evoked whole-cell currents.

Under control conditions in the normal bath, application of 100 μM glutamate alone onto dorsal horn neurons for 2 s induced a large inward current, that rapidly desensitized to a steady state current as shown in Fig. 2Aa. In all four cells tested, the control steady state currents were 25% (25 ± 9%) of peak currents and the decay time constant was 25 ms (25 ± 4 ms). When cells were treated with 100 μM suramin for 2 min and then 100 μM glutamate and 100 μM suramin were applied together, we observed no decrease of the peak and steady state currents (Fig. 2Ab, but also see [8,17]). In

fact, both peak and steady state currents were significantly increased by 16 ± 3% and 78 ± 30%, respectively.

Following treatment with 100 μM suramin, the decay time constants of glutamate-evoked currents were about twice as long as control values (47 ± 11 ms vs. 25 ± 4 ms, $n = 4$). This result was not predicted based on the assumption that suramin inhibited evoked EPSCs by inhibition of AMPA receptors. However, careful examination of the effect of suramin on glutamate-evoked whole-cell currents revealed that the onset and the time to peak of glutamate-evoked whole-cell currents became longer in the presence of suramin (Fig. 2Ad). For all four cells examined, the onset of glutamate-evoked whole-cell currents was slowed by 8 ms (7.5 ± 4, $n = 4$) compare to control. The rise time of glutamate-evoked whole-cell currents also became slower in the presence of suramin. The rise times measured between 10 and 90% of peak amplitude were 13 ± 1 ms in control and significantly prolonged by 60% (20 ± 3 ms) in the presence of 100 μM suramin. As a control we measured the variation of the onset time and the rise time when glutamate was repeatedly applied to a neuron in the absence of suramin (Fig. 2Ae). The range of variation was within ±0.5 ms in the same cell tested with four applications of glutamate (see also Fig. 2Bd). Furthermore, little change of the rise time was found between the trials (Fig. 2Ae). Thus the slowing of onset of glutamate-evoked currents by suramin is a genuine action of the drug and may account for the inhibition of evoked EPSCs shown in Fig. 1.

Fifty micro molar PPADS, which did not show a significant effect on evoked EPSCs (Fig. 1 bottom traces), had little effect on onset and rise time of glutamate-evoked whole-cell currents for the five cells tested (Fig. 2B). The most prominent effect of PPADS was to increase steady state currents (Fig. 2Bb,c). Peak currents were slightly increased by 11% (11 ± 8%) and the steady state currents increased by 70% (70 ± 11%; $n = 5$). There was also a 19% (19 ± 14%) increase in the decay time constants in the presence of 50 μM PPADS. The lack of effect of PPADS on the onset time of glutamate currents may be related to its lack of effect on evoked EPSC amplitude in contrast to the effects of suramin.

We directly addressed whether the suramin-induced decrease in amplitude of the evoked EPSCs was due to its effect on postsynaptic AMPA receptors. This was accomplished by measuring the effects of suramin on the mEPSC amplitude recorded from cultured dorsal horn neurons, based on the assumption that a change in mEPSC amplitude indicates a change in postsynaptic receptor function [9]. mEPSCs were recorded under perforated configuration and analyzed as described previously [12,13]. They were assumed to be mediated by AMPA receptors due to their rapid kinetics and, when tested, their sensitivity to 10 μM CNQX ($n = 5$). mEPSC mean amplitudes ranged from −7.4 to −17.3 pA under control conditions with mean frequencies ranging from 5 to 30 Hz. Fig. 3Aa shows sample traces of mEPSCs obtained from a dorsal horn neuron before, during addition of 100 μM suramin and after wash. When cells were perfused with bath solution containing 100 μM suramin, a decrease in both mEPSC amplitude and frequency was observed (Fig. 3Ab,c). The effect of suramin was reversed after wash (Fig. 3Aa,c). Amplitude histograms of mEPSC recordings are shown in Fig. 3Ab. The leftward shift of the peak toward smaller amplitudes (Fig. 3Ab) suggests that at least some of the decrease in mEPSC frequency is due to the decrease of mEPSC amplitude. For all the cells tested, the mEPSC amplitude decreased to 54% of the control (54 ± 4%, $n = 4$), and the mEPSC frequency decreased to 59% of the control (59 ± 5%, $n = 4$) (Fig. 3Ac). This result is consistent with that of suramin action on evoked EPSCs as shown in Fig. 1. The inhibitory action of suramin on mEPSC amplitude suggests that the suramin effect on evoked EPSC amplitude is due, in large part, to an effect on the postsynaptic AMPA receptors. The decrease in the mEPSC frequency must be due, in part, to a decrease of mEPSC amplitude below the threshold for detection of mEPSCs. In addition, a presynaptic action of suramin causing a decrease of glutamate release probability cannot be excluded.

As a comparison, we examined the effects of PPADS on mEPSCs (Fig. 3B). Fig. 3Ba shows the sample traces of

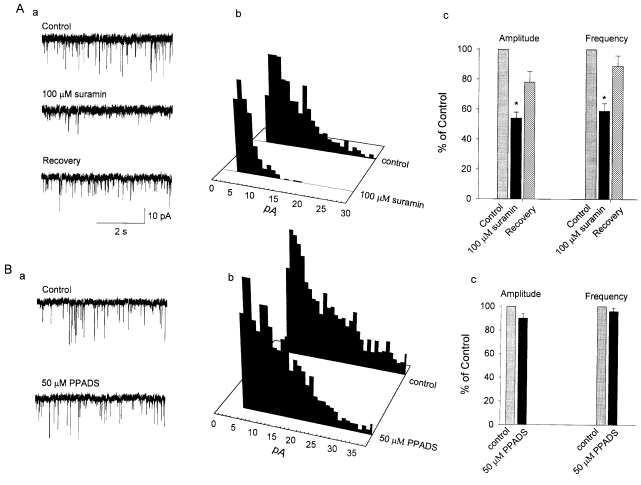

Fig. 3. Effect of suramin and PPADS on mEPSCs recorded from cultured dorsal horn neurons. Inhibitory effects of suramin on mEPSCs. (Aa) Sample traces of mEPSCs before (top), during 100 μM suramin (middle) and after wash off of suramin (bottom). (Ab) Amplitude histogram of mEPSCs recorded before and during 100μM suramin. Bin size is 1 pA. The number of mEPSCs in the bin having the highest number for control is 88, for 100 μM suramin is 97. (Ac) A summary of the change of mEPSC amplitude and frequency before, during 100 μM, and after wash of suramin ($n = 4$). B). Lack of effect of PPADS on mEPSC amplitude and frequency. (Ba) Sample traces of mEPSCs before (top) and during 50 μM PPADS (bottom). (Bb) Amplitude histogram of mEPSCs recorded before and during 50 μm PPADS from the same cell as in Ba. Bin size is 1 pA. The number of mEPSCs in the bin having the highest number for control is 90 and for PPADS is 99. (Bc) A summary of the change of mEPSC amplitude and frequency before and during 50 μM PPADS ($n = 3$). Values are mean ± SEM, $^*P < 0.05$ (Paired Student's t-test).

mEPSCs recorded from a dorsal horn neuron in culture before and after treatment with 50 μM PPADS. The amplitude histogram of mEPSCs for this cell is shown in Fig. 3Bb, indicating little change in the amplitude distribution in the presence of PPADS. In all four cells tested with 50 μM PPADS, there was little inhibition of either mEPSC amplitude or frequency (Fig. 3Bc). This result is consistent with the observation that PPADS had little effect on the evoked EPSCs and further suggests that any effect of suramin on mEPSCs was not mediated by P_{2x} receptors.

The absence of effect of PPADS on the evoked EPSCs found in this study, suggests that this compound does not affect synaptic activation of AMPA receptors. The lack of non-specific effects on synaptic AMPA receptor function and glutamate release makes PPADS a more suitable pharmacological tool for the future exploration of synaptic P_{2x} receptor functions. In contrast, suramin has been shown to have an inhibitory effect on glutamatergic synaptic transmission. Thus, studies using suramin to explore synaptic P_{2x} receptor functions and studies on the in vivo analgesic effects of suramin in cancer patients and [14] animal models [10] should take this and other possible non-specific effects of suramin into account. The inhibitory effect of suramin on glutamatergic synaptic transmission is likely to be due, at least in part, to the slowed onset of synaptic AMPA receptor activation. The mechanism by which suramin slows AMPA current onset is not known, but similar to suramin, the opioid peptide dynorphin and its derivatives were also shown to cause an increase in the rise time of AMPA currents [15]. The opioid suppression of sensory transmission may be partially due to the slowing of AMPA current rise time. This type of synaptic inhibition may provide a novel pharmacological approach for the study of glutamatergic synaptic transmission and its modulation.

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